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THE BRITISH JOURNAL
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EXPERIMENTAL PATHOLOGY

THE MECHANISM OF THE IMMUNITY TO ANTI-PLATELET SERUM.

MARY E. JOHNSTON, B.Sc.

From the Bacteriological Department, Lister Institute, London.

Received for publication July 2nd, 1924.

THE experiments which form the subject of this communication were undertaken with a view to elucidating the causes of the immunity developed in guinea-pigs to anti-platelet serum. Watabiki (1917) found that guinea-pigs could be immunized against anti-platelet serum by one or more sublethal doses of that serum. This immunity requires 10 days for its development, and is shown by the absence of that pathological condition—purpura hæmorrhagica—which, together with the disappearance of platelets from the circulation, is peculiar to the action of an anti-platelet serum *in vivo* (Ledingham, 1913, Ledingham and Bedson, 1915, Bedson, 1921). Watabiki is of the opinion that the platelet antibody has given rise to an antibody, which neutralizes the effect of the platelet antibody, but his view is unsupported by experimental evidence. Bedson (1923), working on this subject, brings forward evidence to show that the immunity is due to the development of an antibody to the proteins of the serum containing the platelet antibody, the serum precipitin thus formed inhibiting the action of the platelet antibody.

These two theories—(1) anti-antibody, (2) precipitin—have been advanced at various times to explain the anti-antisera effects noted by workers on immunological problems. Bordet, in reviewing this subject in his treatise on Immunity (1920), inclines to the anti-antibody explanation, although it must be admitted that his experiments are capable of another interpretation. Pfeiffer and Moreschi (1906) explain their anti-antisera results by the precipitin theory.

In the immunity which animals develop to anti-platelet serum a particularly favourable opportunity presents itself for the solution of this question of anti-antibodies. The existence or non-existence of immunity is shown by the development or non-development of purpura following the inoculation of a purpura-producing dose of anti-platelet serum, a clear-cut end-point facilitating greatly the reading of the experimental results.

Given two anti-platelet sera produced by injecting animals of two different species, *i. e.* goat and rabbit, with the platelets of a third species—guinea-pig—it should be possible to demonstrate clearly whether the immunity which can be produced in guinea-pigs to anti-platelet serum depends on the development of an antibody to platelet antibody, or whether it is merely due to the production of an antibody to the proteins of the serum of the animal species in which the platelet antibody was produced.

EXPERIMENTAL DATA.

The platelets were prepared in the usual way by fractional centrifugalization of citrated guinea-pig's blood.

TABLE I

	J 78			J 81			J 79			J 82			J 84		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
12·6·23	6·8	5,944,000	870,000	7·5	6,872,000	910,000	7·6	5,324,000	700,000	7·3	5,800,000	790,000	7·9	6,608,000	830,000
	1 CC A.P.S. *PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S PREPARED IN GOAT GIVEN I.P.			·5 CC A.P.S PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.		
13·6·23	—	5,432,000	NO PLATELETS SEEN	—	6,104,000	2 PLATELETS PER 1000 RED CELLS	—	4,944,000	NO PLATELETS SEEN	—	4,392,000	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN
	B.T PROLONGED HÆMORRHAGES IN AXILLARY REGION			B.T PROLONGED. SKIN HÆMORRHAGES.			B.T PROLONGED. SKIN HÆMORRHAGES.			B.T. PROLONGED. SKIN HÆMORRHAGES.			B.T. PROLONGED. SKIN HÆMORRHAGES.		
2·7·23	7·2	6,360,000	880 000	7 0	5 848 000	835,000	8·4	5 436,000	650,000	7·0	6,424,000	910,000	6·7	6,400,000	970,000
	·5 CC A.P.S PREPARED IN RABBIT GIVEN I.P.			5 CC A P S PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
3·7·23	—	5,928,000	NO PLATELETS SEEN	—	5 624 000	NO PLATELETS SEEN	8·3	5, 808,000	690,000	—	6,000,000	NO PLATELETS SEEN	—	—	14 PLATELETS PER 1000 RED CELLS
	← GENERALISED PURPURA OF SKIN, STOMACH & GUT WALL. LARGE BLOOD CLOT IN GUT.			SHOWED GENERALISED PURPURA.			KILLED (CHLOROFORM) SHOWED NO SIGNS OF HÆMORRHAGES IN ANY PART OF BODY.			SHOWED GENERALISED FAIRLY SEVERE PURPURA. SOME LARGE HÆMORRHAGIC PATCHES ON SKIN & BODY WALL.			SHOWED NO SIGNS OF PURPURA. →		
	PURPURA			PURPURA			NO PURPURA			PURPURA			NO PURPURA		

CONTROL J83 2·7·23 R/P 5·6 1 CC A.P.S PREPARED IN GOAT GIVEN I.P.
3·7·23 — NO PLATELETS SEEN KILLED. SHOWED PURPURA CHIEFLY SUBCUTANEOUS.
CONTROL J85 2·7·23 R/P 5·6 ·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.
3·7·23 — NO PLATELETS SEEN KILLED. SHOWED PURPURA CHIEFLY SUBCUTANEOUS.

*A.P.S. = ANTIPLATELET SERUM. I.P. INTRAPERITONEALLY, S.C. SUBCUTANEOUSLY. THESE ABBREVIATIONS WILL BE ADHERED TO THROUGHOUT THE VARIOUS TABLES.
B.T. = BLEEDING TIME.

Anti-guinea-pig platelet sera were prepared in the rabbit and the goat, the animals being inoculated intravenously at intervals of 4 to 5 days with the platelets collected from the blood of about 20 guinea-pigs. Ten days after the last inoculation the serum was tested and the animals bled out.

Platelet counts were made by the method fully described by Bedson, (Bedson and Zilva, 1923).

Immunization with Anti-platelet Serum.

Experiment 1.—Guinea-pigs treated with anti-platelet serum prepared in the rabbit were tested 20 days later by anti-platelet serum prepared in the

goat. Similarly, guinea-pigs inoculated with anti-platelet serum prepared in the goat were tested with anti-platelet serum prepared in the rabbit. The results are given in Table I.

J. 78 and J. 81, each given an immunizing dose of anti-platelet serum prepared in the goat, and 20 days later given a test dose of anti-platelet serum prepared in the rabbit, show in both cases disappearance of platelets from the circulation and development of purpura; *no immunity*. J. 79, J. 82 and J. 84, each given an immunizing dose of anti-platelet serum prepared in the rabbit, followed 20 days later in the case of J. 79 by a test dose of anti-platelet serum prepared in the rabbit, and in the case of J. 82 and 84 by a test dose of anti-platelet serum prepared in the goat.

J. 79 shows the development of immunity by no change in platelet count and no production of purpura.

J. 82 shows no immunity by development of typical purpura.

J. 84 shows reduction of platelet, but no purpura.

No immunity, therefore, has been produced in guinea-pigs against anti-platelet serum prepared in the rabbit by inoculations of anti-platelet serum prepared in the goat, nor does anti-platelet serum prepared in the rabbit protect against anti-platelet serum prepared in the goat, although it protects against a second dose of anti-platelet serum prepared in the rabbit.

Further experiments on the same lines as Experiment 1 are tabulated in Tables IIA and IIB.

The results in Table IIA show that the inoculation of anti-platelet serum prepared in the rabbit has not protected the guinea-pigs against a dose of

TABLE IIA

IMMUNITY DEVELOPED TO ANTIPLATLET SERUM PREPARED IN RABBIT AFTER 21 DAYS

	J9			J10			J11			J12			J13			J14		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
11·7·23	5·6	—	—	6·6	—	—	7·5	—	—	7·6	—	—	7·1	—	—	8·7	—	—
	·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.		
12·7·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN
	PIG LOOKS SICK. SKIN HÆMORRHAGES.			B.T. PROLONGED SMALL SKIN HÆMORRHAGES			B.T. PROLONGED. SMALL SKIN HÆMORRHAGES.			SMALL HÆMORRHAGES IN SKIN BEHIND THE EAR.			SMALL HÆMORRHAGES IN SKIN.			SMALL HÆMORRHAGES IN SKIN.		
2·8·23	6·4	—	—	6·2	—	—	7·0	—	—	7·7	—	—	7·1	—	—	7·1	—	—
	1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.		
3·8·23	—	—	NO PLATELETS SEEN	—	—	14 PLATELETS PER 1000 RED CELLS	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	9·3	—	—	8·4	—	—
	SHOWED SUBCUTANEOUS HÆMORRHAGES. ISOLATED HÆMORRHAGES ON GUT & BODY WALL. PURPURA			SHOWED ISOLATED HÆMORRHAGES ON GUT & BLADDER WALL. PURPURA			KILLED (CHLOROFORM) ISOLATED HÆMORRHAGES ON GUT & STOMACH WALL NO SUBCUTANEOUS HÆMORRHAGES. PURPURA			SHOWED HÆMORRHAGES ON GUT WALL & BODY WALL. PURPURA			SHOWED TINY PETECHIÆ ON MESENTERY NO SKIN OF HÆMORRHAGES ANYWHERE ELSE. NO PURPURA			SHOWED TINY PETECHIÆ ON MESENTERY & ONE GUT WALL HÆMORRHAGIC. NO PURPURA		

CONTROL JX 31·7·23 R/P 6·9 1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.
1·8·23 R/P — NO PLATELETS SEEN. ON KILLING SHOWED SEVERE GENERALISED PURPURA.

CONTROL JY 21·7·23 R/P 7·6 ·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.
1·8·23 R/P — NO PLATELETS SEEN. KILLED SHOWED SUBCUTANEOUS HÆMORRHAGES ALSO ON WALL OF INTESTINE, BLADDER & STOMACH.

TABLE II B

IMMUNITY DEVELOPED TO ANTIPLATELET SERUM PREPARED IN GOAT AFTER 21 DAYS.

	J3			J5			J6			J8			J1			J2		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
10·7·23	6·9	—	—	7·6	5 672,000	746,000	6·4	4,712,000	730,000	7·8	—	—	8·3	—	—	7·9	—	—
	1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
11·7·23	—	—	5 PLATELETS PER 1000 RED CELLS	—	5,250,000	NO PLATELETS SEEN	—	4,606,000	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	2 PLATELETS PER 1000 RED CELLS
	B.T. PROLONGED SMALL SKIN HÆMORRHAGES.			B.T. PROLONGED SMALL SKIN HÆMORRHAGES			B.T. PROLONGED SMALL SKIN HÆMORRHAGES			SKIN HÆMORRHAGES BEHIND THE EAR & IN AXILLARY REGION.			PIG LOOKS SICK. BLEEDING FROM NOSE & RECTUM. SKIN HÆMORRHAGES.			B.T. PROLONGED SMALL SKIN HÆMORRHAGES.		
1·8·23	T·T	—	—	8·8	—	—	T·0	5,800,000	820,000	T·5	—	—	8·2	—	—	T·T	—	—
	·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
2·8·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN
	← SEVERE GENERALISED PURPURA. PURPURA			SHOWED SUBCUTANEOUS & BODY WALL HÆMORRHAGES. ONE VERY LARGE HÆMORRHAGE IN INTESTINAL WALL. PURPURA			KILLED (CHLOROFORM) GENERALISED PURPURA. PURPURA			TYPICAL PURPURA PURPURA			SHOWED SMALL SKIN HÆMORRHAGES HÆMORRHAGES ON WALL OF STOMACH & INTESTINE. PURPURA			SHOWED HÆMORRHAGES CHIEFLY ON CÆCUM & BLADDER WALL. PURPURA		

CONTROL JA 1·8·23 R/P 6·9 1CC A.P.S. PREPARED IN GOAT. GIVEN I.P.
2·8·23 —— NO PLATELETS SEEN. KILLED. SHOWED SEVERE PURPURA.

CONTROL JB 1·8·23 R/P 7·6 ·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.
2·8·23 —— NO PLATELETS SEEN. KILLED. SHOWED GENERALISED PURPURA.

TABLE III A.

IMMUNITY DEVELOPED TO ANTIPLATELET SERUM PREPARED IN RABBIT AFTER 14 DAYS.

	J15			J16			J19			J20		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
12·7·23	6·0	—	—	7·7	—	—	7·2	6,024,000	850,000	6·5	6,368,000	580,000
	·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.		
13·7·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	5,404,000	NO PLATELETS SEEN	—	6,568,000	NO PLATELETS SEEN
	B.T. PROLONGED SMALL HÆMORRHAGES ON SKIN.			SKIN HÆMORRHAGES BEHIND THE EAR.			SMALL SKIN HÆMORRHAGES			SMALL SKIN HÆMORRHAGES ON THE BACK.		
26·7·23	7·9	—	—	7·5	—	—	7·8	5,616,000	720,000	6·8	5,878,000	860,000
	1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
27·7·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	5,688,000	3 PLATELETS PER 1000 RED CELLS	—	6,120,000	9 PLATELETS PER 1000 RED CELLS
	← SHOWED SMALL PETECHIÆ ON BODY WALL. ISOLATED GUT HÆMORRHAGES. PURPURA			KILLED (CHLOROFORM) SHOWED HÆMORRHAGES ON BODY WALL DUODENAL WALL. SKIN HÆMORRHAGES. PURPURA			SHOWED SMALL PETECHIÆ ON BODY WALL. PURPURA			SHOWED SOME SMALL PETECHIÆ ON BODY WALL & GUT. PURPURA		

CONTROL J21 31·7·23 R/P 6·9. 1·0 CC A.P.S. PREPARED IN GOAT GIVEN I.P.
1·8·23 R/P —— NO PLATELETS SEEN. KILLED. SHOWED FAIRLY SEVERE PURPURA.

anti-platelet serum prepared in the goat 21 days later. J. 13 and J. 14 show protection developed against anti-platelet serum prepared in the rabbit, as they show practically no reduction in platelets and no true purpura. The minute hæmorrhages on the mesentery, although misleading, can be explained as a purely local effect.

The results given in Table IIB show that guinea-pigs inoculated with anti-platelet serum prepared in the goat develop no immunity against anti-platelet serum prepared in the rabbit. They also in this series of experiments seem to have developed very little immunity against anti-platelet serum prepared in the goat. An individual variation in the development of an immunity to anti-platelet serum was noted by Bedson (*loc. cit.*).

TABLE IIIB

IMMUNITY DEVELOPED IN 14 DAYS TO ANTIPLATELET SERUM PREPARED IN GOAT.

	J 23			J 24			J 25			J 27			J 28			J 21			J 22		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
16·7·23	7	—	—	8·4	—	—	7·1	—	—	7·4	5,836,000	780,000	8·0	6,200,000	776,000	7·1	—	—	7·6	—	—
	1 CC. A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
17·7·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	ONE PLATELET PER 1000 RED CELLS SEEN	—	5,872,000	NO PLATELETS SEEN	—	6,168,000	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN
	PIG LOOKS SICK & LIMP WITH PROLONGED B.T.			HÆMORRHAGES IN SKIN OF ABDOMINAL WALL			HÆMORRHAGES IN SKIN OF BACK			BLEEDING FROM GUMS. HÆMORRHAGES IN SKIN OF BACK & ABDOMINAL WALL			SMALL HÆMORRHAGES IN SKIN SEEN			SMALL SKIN HÆMORRHAGES			B.T. PROLONGED HÆMORRHAGES IN SKIN OF BACK.		
20·7·23	7·2	—	—	6·2	—	—	6·7	—	—	7·8	5,328,000	660,000	8·2	6,284,000	760,000	6·7	—	—	7·8	—	—
	5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.		
21·7·23	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	5,776,000	NO PLATELETS SEEN	—	6,376,000	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN	—	—	NO PLATELETS SEEN
	SHOWED SEVERE GENERALISED PURPURA. PURPURA			SHOWED HÆMORRHAGES IN BODY WALL & BLADDER WALL. PURPURA			SHOWED GENERALISED PURPURA. PURPURA			KILLED (CHLOROFORM) SHOWED SEVERE GENERALISED PURPURA. PURPURA			GENERALISED PURPURA PURPURA			SHOWED SUBCUTANEOUS HÆMORRHAGES & VERY SMALL PETECHIÆ ON BODY WALL GUT & MESENTERY PURPURA			SHOWED SKIN HÆMORRHAGES ALSO SOME ON WALL OF STOMACH & INTESTINES. PURPURA		

CONTROL J 26 30·7·23 R/P 6·9 1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.
31·7·23 — NO PLATELETS SEEN. KILLED. SHOWED SEVERE GENERALISED PURPURA.

Experiment 2.—Tables IIIA and IIIB give results of experiments which are simply a repetition of Experiment 1, except that in this case the interval allowed for the development of immunity was 14 days.

Here, again, anti-platelet serum prepared in the goat does not protect guinea-pigs against anti-platelet serum prepared in the rabbit.

J. 21 and J. 22 appear to have developed no immunity against anti-platelet serum prepared in the goat.

The results given in Table IIIA show that anti-platelet serum prepared in the rabbit does not protect against anti-platelet serum prepared in the goat.

Immunization with Normal Serum.

Experiment 3.—Guinea-pigs were inoculated with a dose of normal serum, and at the end of 3 weeks given a test dose of anti-platelet serum. The results are given in Table IV.

TABLE IV.

IMMUNISATION BY NORMAL SERA AGAINST ANTIPLATELET SERUM.

	J85			J86			J34			J35			J87			J88		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
14·6·23	8·2	6,480,000	790,000	7·0	6,600,000	940,000	—	—	—	—	—	—	—	—	—	—	—	—
	1 CC NORMAL GOAT SERUM GIVEN I.P.			1 CC NORMAL GOAT SERUM GIVEN I.P.			1 CC NORMAL RABBIT SERUM GIVEN I.P.			1 CC NORMAL RABBIT SERUM GIVEN I.P.			1 CC NORMAL RABBIT SERUM GIVEN I.P.			1 CC NORMAL RABBIT SERUM GIVEN I.P.		
5·7·23	7·9	5,864,000	740,000	6·9	6,640,000	960,000	8·1	6,208,000	760,000	7·0	7,112,000	1,000,000	7·1	6,128,000	860,000	7·7	6,320,000	830,000
	·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.			·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.			1 CC A.P.S PREPARED IN GOAT GIVEN I.P.			·5 CC A.P.S PREPARED IN RABBIT GIVEN I.P.		
6·7·23	—	4,700,000	NO PLATELETS SEEN	—	6,500,000	NO PLATELETS SEEN	—	5,600,000	NO PLATELETS SEEN	4·1	6,808,000	160,000	—	—	—	—	6,088,000	3 PLATELETS PER 1000 RED CELLS
	← SHOWED GENERALISED PURPURA.			SUBCUTANEOUS HEMORRHAGES.			KILLED (CHLOROFORM) GENERALISED PURPURA.			NO SIGNS OF HEMORRHAGE. →			DIED DURING THE NIGHT OF 5TH. P.M. SHOWED HEMORRHAGES OF GUT WALL. NO SKIN HEMORRHAGES. PERITONITIS.			KILLED (CHLOROFORM) PURPURIC SPOTS IN SUBCUTANEOUS TISSUE. ISOLATED HEMORRHAGES IN INTESTINAL WALL.		
	PURPURA			PURPURA			PURPURA			NO PURPURA			PURPURA			PURPURA		

CONTROL J C 5·7·23 R/P 5·6 ·5 CC A.P.S. PREPARED IN RABBIT GIVEN I.P.
6·7·23 NO PLATELETS SEEN. KILLED. PURPURA.

CONTROL J D 5·7·23 R/P 7·0 1 CC A.P.S. PREPARED IN GOAT GIVEN I.P.
6·7·23 NO PLATELETS SEEN. KILLED. SHOWED PURPURA CHIEFLY SUBCUTANEOUS.

These results show that normal goat serum does not protect against a dose of anti-platelet serum prepared in the rabbit—J. 85—nor has it produced any immunity against anti-platelet serum prepared in the goat—J. 86.

Normal rabbit serum has developed protection against anti-platelet serum prepared in the rabbit in the case of J. 35, and a slight protection in the case of J. 88, but no protection against anti-platelet serum prepared in the goat—J. 34 and J. 87.

Immunization by a Number of Small Doses of Normal Serum or Anti-platelet Serum.

Experiment 4.—A further series of experiments was carried out, giving normal goat serum or anti-platelet serum at 4 to 5 days' interval, and, 10 days after the last inoculation, a test dose of anti-platelet serum prepared in the rabbit. Unfortunately the anti-platelet serum prepared in the goat had unaccountably lost strength in the few days elapsing between testing its purpura-producing power and the test doses, so that the control showed disappearance of platelets, but no purpura.

Only the results where anti-platelet serum prepared in rabbit was used in the test dose, were of value.

In these experiments inoculations were carried out subcutaneously to avoid any confusing local effect.

These results (Table V) simply confirm earlier experiments, showing that even repeated small doses, either of normal goat serum or of anti-platelet serum prepared in the goat, do not protect guinea-pigs against anti-platelet serum prepared in the rabbit. In the case of J. 36 no purpura had developed, although all the platelets had disappeared from the circulation.

A summary of the results in Tables I–V shows:

1. Anti-platelet serum prepared in the rabbit does not protect against

anti-platelet serum prepared in the goat, but does protect against anti-platelet serum prepared in the rabbit.

2. Anti-platelet serum prepared in the goat does not protect against anti-platelet serum prepared in the rabbit, but to a small extent protects against anti-platelet serum prepared in the goat.

3. Normal rabbit serum protects against anti-platelet serum prepared in the rabbit, but not against anti-platelet serum prepared in the goat.

4. Normal goat serum does not protect against anti-platelet serum prepared in the rabbit.

TABLE V.

IMMUNISATION BY REPEATED SMALL DOSES OF NORMAL GOAT SERUM OR ANTI-PLATELET SERUM PREPARED IN GOAT.

DATE	J 35	J 36	J 41	J 42
10·9·23	0·5 cc NORMAL GOAT SERUM. I.P.		0·2 cc A.P.S. PREPARED IN GOAT. I.P.	
14·9·23	" "	" "	0·3 cc " "	" "
18·9·23	" "	" "	0·5 cc " "	" "
21·9·23	" "	" "	0·5 cc " "	" "
25·9·23	" "	" "	0·3 cc " "	" "
2·10·23	" "	" "	0·5 cc " "	" "
	R/S	R/S	R/S	R/S
11·10·23	6·5	6·8	8·9	7·5
11·10·23	<----1·0 cc A.P.S. PREPARED IN RABBIT S.C.---->			
12·10·23	<-------NO PLATELETS SEEN IN PERIPHERAL BLOOD---------->			
	<----------KILLED (CHLOROFORM)---------->			
	SHOWED GENERALISED PURPURA.	NO HAEMORRHAGES SEEN.	SHOWED HAEMORRHAGES IN SUBCUTANEOUS TISSUE & IN STOMACH & INTESTINAL WALL.	SHOWED GENERALISED PURPURA.
	PURPURA	NO PURPURA	PURPURA	PURPURA

CONTROL. 11·10·23 R/S 8·5. RECEIVED 1·0 cc A.P.S. PREPARED IN RABBIT S.C.
12·10·23 NO PLATELETS. KILLED. SHOWED GENERALISED PURPURA.

Immunity Produced by Anti-platelet Serum to Anti-red-cell Serum, both Prepared in the Same Animal (Rabbit).

On account of the difficulty experienced in preparing a satisfactory anti-platelet serum in the goat and the apparent failure of the guinea-pig to react to normal goat serum with the production of antibodies, it was decided to approach this problem from another angle. It has been shown that the absorption of anti-platelet serum with red corpuscles removes the antibody for red cells, leaving intact the specific antibody for platelets (Sacerdotti, 1908, Bedson 1922), and further, that it is in virtue of the specific antibody for platelets that anti-platelet serum reacts *in vivo* with the production of purpura, (Bedson, 1922). Now if guinea-pigs be immunized with absorbed anti-platelet serum, then, on the assumption that the protection is due to the development of an anti-antibody, one would expect them to be protected against a test dose of anti-platelet serum, but to react to the introduction of a dose of anti-red-cell serum with the characteristic rapid fall in the red-cell count and the sick and anæmic appearance of the animal. On the other hand, should the immunity be due to the development of an antibody to the serum proteins, then the guinea-pigs should be protected both against anti-platelet and anti-red-cell serum, since both sera were prepared in the same animal species—the rabbit. This was put to the test in the following experiment:

Experiment 5.—1·0 c.c. of anti-platelet serum was absorbed for 2 hours at 37° C. with 4·0 c.c. of a 50 per cent. red-cell suspension. The red cells were

then removed by centrifugalization and the supernatant fluid used for inoculation. The dilution of the anti-platelet serum was then approximately 1 : 3, so that a dose of 0·6 c.c. represented 0·2 c.c. undiluted serum. Freshly absorbed anti-platelet serum was used for each inoculation and the degree of absorption estimated on each occasion.

The results are tabulated below in Tables VI and VII.

TABLE VI.

IMMUNISATION BY SMALL DOSES OF ABSORBED ANTIPLATELET SERUM.

	R1			R2			R3			R4			R5			R6		
DATE	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS	R/P	RED CELLS	PLATELETS
23·1·24	T·1	5,504,000	T70,000	T·5	6,664,000	880,000	T·8	6,480,000	830,000	8·3	6,290,000	T30,000	T·9	6,296,000	T90,000	T·1	6,328,000	880,000
	·2 CC ABSORBED A.P.S. GIVEN I.P. →																	
24·1·24	—	5,130,000	3 PLATELETS PER 1000 RED CELLS	—	5,984,000	NO PLATELETS SEEN	—	5,408,000	NO PLATELETS SEEN	—	6,328,000	NO PLATELETS SEEN	—	4,368,000	NO PLATELETS SEEN	—	5,552,000	NO PLATELETS SEEN
30·1·24	5·6	3,088,000	550,000	6·6	3,392,000	810,000	4·2	3,904,000	320,000	6·8	4,168,000	610,000	5·6	3,848,000	540,000	3·9	3,840,000	980,000
	·2 CC ABSORBED A.P.S. GIVEN I.P. →																	
31·1·24	—	3,288,000	6 PLATELETS PER 1000 RED CELLS	20·9	4,881,000	230,000	31·2	4,072,000	130,000	—	4,488,000	NO PLATELETS SEEN	—	4,080,000	NO PLATELETS SEEN	18·8	4,056,000	210,000
4·2·24	4·9	3,224,000	650,000	6·8	3,288,000	T70,000	6·8	4,688,000	680,000	T·6	3,200,000	680,000	5·9	4,136,000	T00,000	8·5	5,416,000	630,000
	·3 CC ABSORBED A.P.S. GIVEN I.P. →																	
5·2·24	68·8	4,136,000	60,000	22·1	6,088,000	270,000	64·3	6,050,000	94,000	19·3	4,984,000	250,000	6·1	4,256,000	T00,000	31·2	5,160,000	630,000
11·2·24	6·3	4,360,000	690,000	5·6	4,952,000	880,000	6·T	5,800,000	860,000	6·3	5,680,000	840,000	T·2	5,346,000	T40,000	T·5	5,952,000	790,000
	·3 CC ABSORBED A.P.S. GIVEN I.P.																	
12·2·24	—	4,360,000	NO PLATELETS SEEN	31·T	4,792,000	150,000	15·9	6,128,000	380,000	23·1	5,976,000	250,000	43·6	5,176,000	110,000	43·5	5,688,000	130,000
22·2·24 4 P.M.	6·8	5,080,000	T40,000	6·8	6,568,000	960,000	T·6	6,760,000	810,000	8·2	6,448,000	T80,000	T·4	5,464,000	T30,000	T·6	6,760,000	880,000
	·3 CC UNABSORBED A.P.S. GIVEN I.P.			·3 CC ANTI RED CELL SERUM GIVEN I.P.			·3 CC ANTI RED CELL SERUM GIVEN I.P.			·3 CC ANTI RED CELL SERUM GIVEN I.P.			3 CC UNABSORBED A.P.S. GIVEN I.P.			·3 CC UNABSORBED A.P.S. GIVEN I.P.		
23·2·24 10 A.M.	14·9	5,328,000	350,000	T·5	5,868,000	T80,000	T·T	6,712,000	870,000	T·9	6,192,000	T80,000	34·T	5,156,000	140,000	11·9	6,880,000	570,000
	← NO HÆMORRHAGES ANYWHERE. NO PURPURA			PIG LOOKED PERFECTLY NORMAL. NO FALL IN RED CELLS			KILLED (CHLOROFORM) PIG QUITE NORMAL. NO FALL IN RED CELLS			PIG SHOWED NORMAL FINDINGS EXCEPT THAT SPLEEN WAS DARK, RED & LARGE. NO FALL IN RED CELLS			SHOWED NO HÆMORRHAGES ANYWHERE. NO PURPURA			PIG QUITE NORMAL NO HÆMORRHAGES. NO PURPURA →		

CONTROL R9 22·2·23 RED CELLS 6 728 000 2 P.M. ·3 CC ANTI RED CELL SERUM GIVEN I.P.

23·2·23 RED CELLS 2 952 000 10 A.M. KILLED SHOWED FREE BLOOD IN PERITONEUM & BLADDER. ORGANS PALE & BLOODLESS, SPLEEN LARGE & DARK RED.

CONTROL R8 21·2·23 R/P 6·2 ·3 CC A.P.S. GIVEN I.P.

22·2·23 — NO PLATELETS SEEN. KILLED. SHOWED GENERALISED PURPURA.

The results in Table VI show quite clearly that the guinea-pigs, as well as developing an immunity to anti-platelet serum, have also developed an immunity to anti-red-cell serum. This cannot be due to the production of an antibody against the red cell antibody, since all red cell antibody was removed from the anti-platelet serum by absorption against red cells. Remembering that a dose of anti-platelet serum contains two factors, namely, platelet antibody and normal rabbit serum proteins, is it not possible that the normal rabbit serum fraction gives rise to an antibody response in the guinea-pig? A second dose of normal rabbit serum, which is contained in both the anti-platelet and anti-red-cell serum, results in an antigen antibody

complex being formed, which exerts some inhibitory action on the union of platelet and red cell antibody with their antigens.

TABLE VII.

DEGREE OF ABSORPTION OF ANTIPLATELET SERUM. (AGAINST 2% SUSPN OF RED CELLS).

	FINAL DILUTION OF SERUM AFTER ABSORPTION.						
DATE.	1/12	1/24	1/48	1/96	1/192	1/384	
23·1·24.	±	−	−	−	−	−	AFTER 2 HRS AT 37° C.
	−	−	−	−	−	−	AFTER 24 HRS AT ROOM TEMPERATURE.
30·1·24.	−	−	−	−	−	−	
	−	−	−	−	−	−	
4·2·24.	∓	−	−	−	−	−	
	±	−	−	−	−	−	
11·2·24.	±	−	−	−	−	−	
	+	±	−	−	−	−	

+ = SLIGHT AGGLUTINATION.
± = TRACE OF AGGLUTINATION.
∓ = SLIGHT TRACE OF AGGLUTINATION.

DISCUSSION.

Evidence in this paper seems to negative the anti-antibody theory as an explanation of the immunity developed in animals against anti-platelet serum. The specificity of the platelet antibody produced in either the rabbit or goat is shown by its effect on the animal *in vivo*; and if this antibody plays the part of antigen in the production of an anti-antibody it should give rise to a specific anti-antibody. The foregoing experiments show that this is not the case, and that platelet antibody produced from one source is incapable of protecting the animal against platelet antibody produced from a second source. The so-called "anti-antibodies" "bear the seal of their place of origin"—in fact the immunity developed depends upon the normal serum fraction of the anti-platelet serum. Bordet (*loc. cit.*), in his work with hæmolytic sera, and Pfeiffer and Friedberger (1903), working with anti-cholera serum, both recognize the close connection between the source of origin of the antibody and the production of the so-called anti-antibody. Bordet merely suggests that the anti-antibody is unique in that it distinguishes the place of origin of the antibody and the nature of the antigen producing it.

It is obvious from these experiments that anti-platelet serum prepared in the rabbit is much more effective than anti-platelet serum prepared in the goat in producing an immunity. A dose of the former serum very rarely fails to protect against a second dose, while in the latter case the immunity is slight, and sometimes does not develop at all. On the anti-antibody theory these results would be difficult to explain, for why should the platelet antibody give rise to such a different response in the same animal, *i. e.* guinea-pig? In explaining the immunity developed by the precipitin theory, there are two separate antigens to be considered in connection with anti-platelet serum prepared either in the rabbit or goat, namely, the proteins in rabbit serum and the proteins in goat serum.

The same animal varies greatly in its production of antibody to different antigens, and possibly the guinea-pig produces an antibody more readily to the

rabbit serum proteins than to the goat serum proteins. *In vitro* experiments on the precipitating power of the serum of immunized guinea-pigs with rabbit or goat serum have not been satisfactory in demonstrating more than a trace of precipitin to be present.

Repeated small doses of anti-platelet serum prepared in the goat or of normal goat serum did not seem to increase the immunity, but further work on the optimum conditions for the development of a maximum precipitating titre might clear up this matter. It would also be interesting to repeat these experiments with an anti-platelet serum prepared in a third animal species, to the normal serum of which the guinea-pig is able to respond with the production of antibodies.

The existing knowledge of the nature of antibodies is so slight that any explanation of the inhibiting action exerted by the specific precipitate must be merely a suggestion. It is known that antibodies are intimately associated with the proteins of the serum; in fact, it has been impossible to entirely free them from these proteins. This being so, it is not unreasonable to suppose that the union of the precipitin with the serum proteins of the anti-platelet serum results in the formation of a complex, in which the platelet antibody is entangled and thus rendered innocuous. Recent work by Opie (1924) is of interest in this respect. He has shown that, contrary to what pertains in the normal animal, the specific antigen injected intracutaneously into immunized animals remains entirely localized and never appears in the circulation. It is possible, therefore, that in the immunized guinea-pigs the anti-platelet serum is not absorbed from the site of inoculation, and thus is unable to act on the platelets and capillary endothelium.

This question of anti-antibodies is of no mere academic importance; its bearing on serum therapy is obvious. The available evidence on these questions is conflicting, and further study along these lines is desirable.

CONCLUSIONS.

1. The anti-antibody theory put forward to explain the anti-antisera effects in immunological phenomena is unsupported by experimental evidence.
2. Guinea-pigs immunized with anti-platelet serum prepared in one animal do not develop an immunity to the same antibody prepared in an animal of another species.
3. Guinea-pigs can be immunized against platelet antibody by the inoculation of normal serum from the same species of animal as that in which the platelet antibody is prepared.
4. So called "anti-antisera" effects are not specific to the antibody supposed to produce the effect, but are dependent on the source of origin of the antibody and can be explained by the precipitin theory.
5. The results of experiments of other workers can be explained simply on the precipitin theory.

I wish to thank Prof. Ledingham for his kindness in allowing me to carry out this work in the Bacteriological Department of the Lister Institute, and Dr. Bedson for suggesting this line of work to me.

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THE BACTERICIDAL PROPERTY OF MILK.

FINN S. HANSSEN.

From Dr. med. F. G. Gade's Pathological Institute, Bergen, Norway.

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IN spite of the fact that milk may serve as a medium for the cultivation of bacteria, fresh milk has been found to possess bactericidal or antiseptic qualities. The opinions of the various authors on this question differ. Some deny the existence of such properties, but most have found fresh milk during the first hours after milking to be capable of reducing the number of bacteria with which it has been infected. Further experiments being necessary, the following investigation may be of some interest.

The milk samples used were taken as sterile as possible. In order to clean out mechanically the lactiferous ducts of the cow, the first portions during the milking were thrown away, and the samples of milk to be examined were then obtained by milking directly into sterile test-tubes. The experiments were started 3–4 hours later. In this way very clean samples of milk were procured. They often proved sterile at the beginning of the experiment, and even after 3–4 hours' of incubation at 37° C. they frequently remained sterile, or rarely gave a number of bacteria amounting to 1000 per c.c. But after 6–8 or 24–30 hours' incubation the ordinary milk bacteria usually increased greatly, giving countless colonies on agar plates.

The experiments have been performed in the following way:

Portions of the milk were heated in a water-bath to 65°, 70°, 75° or 100° C. for 15 mins. or to 63° C. for 30 mins. 1 c.c. from each of these differently treated portions was distributed into different sterile test-tubes, and infected with known quantities of *B. typhosus* or *B. paratyphosus B.* The infecting dose in each experiment was always the same, varying from 1/16,000 to 1/64,000 loop in the different experiments.

The loop used had a diameter of about 2 mm. The cultures were 24 hours' slant agar cultures.

The fractions of a loop were obtained by suspending 1 loop in sterile physiological saline and then further diluting this emulsion, until 1 drop could be calculated to represent 1/16,000 or 1/64,000 loop. The milk was then infected by dropping one drop to the bottom of the test-tubes.

In each experiment the growth of the microbes has been controlled at different time-intervals, by adding two drops of the contents of each tube to a tube of fluid agar and pouring out to plates.

1. Immediately after the infection such plate cultures were prepared from all tubes, infected as well as uninfected. The first half of these plates served as control of the infecting dose to show that the infection of the tubes was homogeneous at the beginning of the experiment. The second half served as a control of the natural bacterial flora of the uninfected milk at the beginning of the experiment.

2. Then followed incubation at 37° C. for 3–4 hours, when the test-tubes were removed from the incubator, moderately shaken, and the contents of each tube further emulsified by drawing them up and blowing them out through sterile pipettes. Another set of plate agar cultures was then prepared by adding another two drops of the contents of each tube to fluid agar.

3, 4. The test-tubes were replaced in the incubator. After 6–8 or 24–30 hours a third and fourth set of plate agar cultures were prepared in the same way as described above.

All plates were incubated for 24–48 hours, and the number of colonies appearing directly counted. Plates with more than 100 colonies were calculated approximately, and compared with "standard" agar plates where the number of colonies had previously been accurately counted, and afterwards kept well closed in an ice-box.

We have considered the experiment positive in regard to showing bactericidal properties when the number of colonies on agar plates at the beginning of the experiment was reduced from 1000 or more to 100 or less after 3–4 hours' incubation at 37° C.

About 70 samples of milk have been examined by this method. The general result is that when fresh milk is infected with *B. typhosus* or *B. paratyphosus B.* there will very often take place a considerable reduction in the number of colonies.

Table I demonstrates an experiment with distinct bactericidal qualities:

The samples of milk were procured 3–4 hours before examination. Different portions of milk were tested: (1) Left fresh; (2) heated to 63° C. for 30 mins.; (3) heated in boiling water for 15 mins. Infecting dose, 1/16,000 loop of a 24 hours' slant agar culture of *B. typhosus*. Quantity of milk in each tube 1 c.c. Incubated at 37° C. for 3 hours and 32 hours.

In Table I tubes 1–6 represent the infected tubes, 7–9 the controls. In column "e" plates 1–6 are the evidence that all tests have been homogeneously infected at the beginning of the experiment, while plate 7 in column "e" shows the very low number of bacteria in the fresh uninfected milk. In column "f" is found the situation after 3 hours' incubation at 37° C. The reduction of colonies in fresh and pasteurized milk is well marked as compared with boiled milk and the corresponding plates in column "e." A slight increase in the number of bacteria in fresh milk has taken place.

Table II shows how the bactericidal properties are influenced by heating.

The experiment was started 3 hours after milking. Different portions of milk were heated in a water-bath to 65°, 70°, 75° and 100° C. for 15 mins. Quantity of milk in each tube 1 c.c. Infecting dose 1/16,000 loop of 24 hours' slant agar culture of *B. typhosus*. Incubated at 37° C. for 3 hours and 24 hours.

Tubes 1–5 have been infected. Tubes 6–10 were left uninfected. Column "e" is the control of the homogeneous infection in tubes 1–5, and shows the low bacterial index in fresh milk (tube 6) at the beginning of the experiment.

TABLE I.—*Experiment XX.*

a. Test tube No.	b. Quantity of milk.	c. Condition of the milk.	d. Infecting dose of *B. typhosus*.	e. Number of colonies on agar plates. At the beginning of the experiment.	f. Number of colonies on agar plates. After 3 hours' incubation.	g. Number of colonies on agar plates. After 32 hours' incubation.
1	1 c.c.	Fresh	1/16,000 loop	1000–2000	2	Many thousands
2	1 ,,	Heated to 63° C. for 30 mins.	1/16,000 ,,	1000–2000	0	0
3	1 ,,	Boiled for 15 mins.	1/16,000 ,,	1000–2000	1000–2000	Many thousands
4	1 ,,	Fresh	1/32,000 ,,	1000–2000	0	Not examined
5	1 ,,	Heated to 63° C. for 30 mins.	1/32,000 ,,	1000–2000	0	,, ,,
6	1 ,,	Boiled for 15 mins.	1/32,000 ,,	1000–2000	1000–2000	,, ,,
7	1 ,,	Fresh	Non-infected	0	4	Many thousands
8	1 ,,	Heated to 63° C. for 30 mins.	,,	0	0	0
9	1 ,,	Boiled for 15 mins.	,,	0	0	0

TABLE II.—*Experiment XXVIII.*

a. Test-tube No.	b. Quantity of milk.	c. Condition of the milk.	d. Infecting dose of *B. typhosus*.	e. Number of colonies on agar plates. At the beginning of the experiment.	f. Number of colonies on agar plates. After 3 hours' incubation.	g. Number of colonies on agar plates. After 24 hours' incubation.
1	1 c.c.	Fresh	1/16,000 loop	1000–2000	0	Many thousands
2	1 ,,	Heated to 65° C. for 15 mins.	1/16,000 ,,	1000–2000	0	,, ,,
3	1 ,,	Heated to 70° C. for 15 mins.	1/16,000 ,,	1000–2000	9	0
4	1 ,,	Heated to 75° C. for 15 mins.	1/16,000 ,,	1000–2000	Many thousands	Many thousands
5	1 ,,	Boiled for 15 mins.	1/16,000 ,,	1000–2000	,, ,,	,, ,,
6	1 ,,	Fresh	Non-infected	0	0	0
7	1 ,,	Heated to 65° C. for 15 mins.	,,	0	0	0
8	1 ,,	Heated to 70° C. for 15 mins.	,,	1	0	0
9	1 ,,	Heated to 75° C. for 15 mins.	,,	0	0	0
10	1 ,,	Boiled for 15 mins.	,,	0	0	0

TABLE III.—*Experiment XXX.*

a.	b.	c.	d.	e.	f.	g.	h.	i.	k.	l.
				Number of colonies on agar plates.						
Test-tube No.	Quantity of milk.	Condition of the milk.	Infecting dose of *B. typhosus*.	At the beginning of the experiment.	After 15 minutes' incubation.	After 30 minutes' incubation.	After 1 hour's incubation.	After 2 hours' incubation.	After 3 hours' incubation.	After 8 hours' incubation.
1	2 c.c.	Fresh	2/16,000 loop	1000–2000	1000–2000	3	3	1	0	1000–2000
2	2 „	Heated to 70° C. for 15 mins.	2/16,000 „	1000–2000	1000–2000	300	65	2	3	3
3	2 „	Boiled for 15 mins.	2/16,000 „	1000–2000	1000–2000	1000–2000	1000–2000	1000–2000	1000–2000	Many thousands
4	1 „	Fresh	Non-infected	0	0	—	—	—	—	1000–2000
5	1 „	Heated to 70° C. for 15 mins.	„	0	0	—	—	—	—	0
6	1 „	Boiled for 15 mins.	„	0	0	—	—	—	—	0

Column "f" gives the situation after 3 hours' incubation at 37° C. In fresh milk and milk heated up to 70° C. the reduction of colonies is well marked, but no reduction took place in milk heated to 75° C. for 15 min., nor in boiled milk. Uninfected control milk samples remained in this experiment sterile.

The following experiment (*Table III*) shows that the reduction of colonies takes place rather rapidly after infection of the milk tests.

The experiment was started 3–4 hours after milking. One portion was left fresh. Other portions were heated to 70° and 100° C. The quantity of milk in each test-tube, 2 c.c. Infecting dose 2/16,000 loop of 24 hours' plate agar culture of *B. typhosus*. Experiment kept in water-bath at 37° C.

Column "e" proves the homogeneous infection of the infected tubes (Nos. 1–3) and the low number of bacteria in the fresh milk at the beginning of the experiment. 15 minutes after the infection of the tubes (column "f") the situation was unchanged in all tubes. After 30 minutes (column "g") the reduction of colonies was distinct in the fresh milk and traceable in the milk heated to 70° C. It will be noticed that in the fresh milk the maximum reduction is practically reached after 30 minutes. In the milk heated to 70° the maximum reduction was not reached until 2 hours after the infection of the tests.

We have hitherto only looked at the conditions after 3–4 hours' incubation. From a hygienic point of view it is of interest to examine if the reduction of colonies demonstrated lasts when incubation is prolonged. In Table II, column "g," is found the result of 24 hours' incubation. In spite of the indisputable reduction after 3 hours' incubation, countless colonies appeared on plates from fresh milk heated to 65° C. after 24 hours' incubation. In this case, as an exception, the uninfected fresh milk remained sterile for 24 hours. As a rule, the reduction of colonies is only a temporary one. Usually the uninfected fresh milk shows a heavy growth after 24 hours' incubation at 37° C. For this reason it is often difficult to give an exact account of the numerical presence of the colonies of *B. typhosus* after 24 hours' incubation.

Sometimes (*cf.* Table I, column "g," and Table III, column "i") we have found countless colonies on the plates from infected and uninfected fresh milk, but no or only a few colonies on the plate from the previously pasteurized and then typhoid-infected milk, after prolonged incubation. We consider it probable that this means that *B. typhosus* is more sensitive to the bactericidal properties in milk than the ordinary milk bacteria.

Table IV.—Also when kept at the temperature of the room, fresh milk is found to be possessed of bactericidal properties.

Sample of milk examined 4 hours after milking. Different portions of the milk were heated in a water-bath for 15 mins. to 65° and 100° C.; another portion was left fresh. 1 c.c. milk in each tube. Infecting dose 1/64,000 loop of a 24 hours' slant agar culture of *B. typhosus*.

Infected tubes 1–3, column "e," prove the homogeneous infection in tubes 1–3, and show the very small number of bacteria in fresh milk at the beginning of the experiment. Column "f" shows the well-marked reduction of colonies in the fresh and pasteurized milk after 5 hours, as contrasted with the absence of reduction in the boiled sample.

Table V.—In contra-distinction to some other authors, we have also found *B. paratyphosus B.* sensitive to the bactericidal properties in milk; but

TABLE IV.—*Experiment XXXVI.*

a. Test-tube No.	b. Quantity of milk.	c. Condition of the milk.	d. Infecting dose of *B. typhosus*.	e. Number of colonies on agar plates. At the beginning of the experiment.	f. Number of colonies on agar plates. After 5 hours' incubation at the temperature of the room.
1	1 c.c.	Fresh	1/64,000 loop	1000-2000	0
2	1 „	Heated to 65° C. for 15 mins.	1/64,000 „	1000-2000	0
3	1 „	Boiled for 15 mins.	1/64,000 „	1000-2000	1000-2000
4	1 „	Fresh	Non-infected	0	0
5	1 „	Heated to 65° C. for 15 mins.	„	0	0

apparently not all strains are sensitive to the same degree. With one of our laboratory strains (called "Aasene") 7 experiments were performed showing no reduction of colonies. Another strain (called "Odda") was found to be sensitive to the bactericidal properties of milk. In the following experiment the same samples of milk were examined by means of *B. typhosus*, *B. paratyphosus B.* (Odda) and *B. paratyphosus B.* (Aasene).

The milk was examined 4 hours after milking. Different portions were left fresh or heated for 15 mins. in a water-bath to 65° and 100° C., 1 c.c. milk in each tube. Infecting dose 1/16,000 loop of 24 hours' agar cultures of *B. typhosus* and *B. paratyphosus B.* strains "Odda" and "Aasene."

Test-tubes 1-3 were infected with *B. typhosus*. Test-tubes 4–6 with *B. paratyphosus B.* (Odda). Test-tubes 7–9 with *B. paratyphosus B.* (Aasene). Test-tubes 10 and 11 were left uninfected, showing the low number of bacteria in fresh milk at the beginning of the experiment (column "g"). Column "g" also is the control for the homogeneous infection of tubes 1–9. Column "h" gives the situation after 4 hours' incubation. We find a well-marked reduction of the colonies of *B. typhosus* as well as a reduction of the colonies of *B. paratyphosus B.* (Odda). But no reduction has taken place in the tubes infected with *B. paratyphosus B.* (Aasene). The reduction of colonies in this experiment is not complete, partly owing to the growth of ordinary milk bacteria (*cf.* plate from tube 10, column "h," with 100 colonies).

After 32 hours' incubation countless colonies were found even in pasteurized non-infected milk.

Table VI.—The last experiment demonstrates that also at the temperature of the room a reduction of the colonies takes place when the milk is infected with a sensitive strain of *B. paratyphosus B.*

The samples of milk were procured 4 hours before examination. One portion of the milk was left fresh; other portions were heated in a water-bath to 63° C. for 30 mins., and to 100° C. for 15 mins. Infecting dose 1/32,000 loop of a 24 hours' old agar culture of *B. paratyphosus B.* (Odda).

In column "e" are found the control plates of the homogeneous infection of each tube at the beginning of the experiment, and a control of the low

TABLE V.—*Experiment XLVII.*

TABLE V.—*Experiment XLVII.*

a. Test-tube No.	b. Quantity of milk.	c. Condition of the milk.	d. Infecting dose of— *B. typhosus.*	e. Infecting dose of— *B. paratyphosus B.* Odda.	f. Infecting dose of— *B. paratyphosus B.* Aasene.	g. Number of colonies on agar plates. At the beginning of the experiment.	h. Number of colonies on agar plates. After 4 hours' incubation at 37° C.	i. Number of colonies on agar plates. After 32 hours' incubation at 37° C.
1	1 c.c.	Fresh	1/16,000 loop	—	—	1000–2000	100	Many thousands
2	1 „	Heated to 65° C. for 15 mins.	1/16,000 „	—	—	1000–2000	100	„ „
3	1 „	Boiled for 15 mins.	1/16,000 „	—	—	1000–2000	1000–2000	„ „
4	1 „	Fresh	—	1/16,000 loop	—	1000–2000	120	„ „
5	1 „	Heated to 65° C. for 15 mins.	—	1/16,000 „	—	1000–2000	0	„ „
6	1 „	Boiled for 15 mins.	—	1/16,000 „	—	1000–2000	Many thousands	„ „
7	1 „	Fresh	—	—	1/16,000 loop	1000–2000	1000–2000	—
8	1 „	Heated to 65° C. for 15 mins.	—	—	1/16,000 „	1000–2000	Many thousands	—
9	1 „	Boiled for 15 mins.	—	—	1/16,000 „	1000–2000	„ „	—
10	1 „	Fresh	Non-infected	Non-infected	Non-infected	0	100	Many thousands
11	1 „	Heated to 65° C. for 15 mins.	„	„	„	0	0	„ „

TABLE VI.—*Experiment LII.*

a. Test-tube No.	b. Quantity of milk.	c. Condition of the milk.	d. Infecting dose of *B. paratyphosus B.* Odda.	e. Number of colonies on agar plates. At the beginning of the experiment.	f. Number of colonies on agar plates. After 3 hours' incubation at the temperature of the room.	g. Number of colonies on agar plates. After 8 hours' incubation at the temperature of the room.	h. Number of colonies on agar plates. After 25 hours' incubation at the temperature of the room.
1	1 c.c.	Fresh	1/32,000 loop	1000–2000	1000–2000	ca. 1000	ca. 500
2	1 „	Heated to 63° C. for 30 mins.	1/32,000 „	1000–2000	1000–2000	ca. 1000	0
3	1 „	Boiled for 15 mins.	1/32,000 „	1000–2000	1000–2000	Many thousands	Many thousands
4	1 „	Fresh	Non-infected	0	0	50	ca. 500
5	1 „	Heated to 63° C. for 30 mins.	„	0	0	0	0
6	1 „	Boiled for 15 mins.	„	0	0	0	0

bacterial index of the fresh uninfected milk. After 3 hours no noticeable reduction has taken place, and even after 8 hours only a slight sign of reduction is traceable. After 25 hours the reduction is indisputable, in spite of the presence of 500 colonies on the plate from test-tube 1. These 500 colonies evidently represent normal milk bacteria, because the same number of bacteria is found on the plate from non-infected fresh milk (tube 4, column "h").

The agar plate from pasteurized infected milk was sterile when infected and non-infected fresh milk gave 500 colonies. Similar relations have sometimes been found (as mentioned above regarding *B. typhosus*), and we have thought it likely that this strain of *B. paratyphosus B.* is also more sensitive to the bactericidal qualities of milk than the ordinary milk bacteria.

Experiments have been carried out in samples of milk 24 hours after milking. The milk during this time was placed in an ice-box, and proved to be still in possession of bactericidal properties.

The examples referred to above might give a false idea of the bactericidal qualities in milk, if we did not add that these qualities have been found to vary greatly at different times of the year.

Our experiments were commenced in January, 1922. Sixteen samples of milk were examined. Two of these were found in possession of well-marked bactericidal properties. In the remaining 14 tests no bactericidal properties or only slight traces of them could be found.

In September–October the experiments were continued. Nineteen tests were examined, and all of them were found in possession of well-marked bactericidal properties.

In November–December, 1922, 29 samples were examined. Twelve gave marked reduction of colonies, but in 17 no bactericidal properties, or only slight ones, could be found. The experiments were carried out in the same way all the time. The same strains of *B. typhosus* and *B. paratyphosus B.* were used. The samples of milk in all experiments—except 10—were taken from the same cow. It is very probable that this variability of the bactericidal properties of milk has a close relation to variations in the food given to the cow. In September–October the cow, besides strong food, received fresh grass, turnip, cabbage. In January, February, and November–December (periods when the milk proved to be in possession of only very feeble bactericidal properties or none at all) the cow received no fresh grass, but, besides strong food, only a sort of ensilage of grass, which is common as food for cattle in West Norway in the winter time. This ensilage is prepared by putting cut grass into large hay-stacks, under pressure, in which for some months a sort of fermentation takes place, a fermentation which is found especially to produce lactic acid and, partly, acetic and butyric acids and alcohol. Finally, we should add that our cow had her last calf in the middle of July. In no period of our experiments, therefore, have our milk-samples had the character of colostrum.

REFERENCES IN THE LITERATURE.

The nature of the bactericidal properties of milk has been interpreted in different ways. Basenau (1895) and Stocking (1904) mentioned a slight reduction of colonies in fresh milk, but thought that this reduction was caused

by the inoculation of the bacteria into a new medium, and due to "the natural dropping out of those species which do not find milk a suitable medium in which to develop" (Stocking). Behring (1904) and Moro (1907) thought that the bactericidal properties were due to alexin in the milk, arguing that all bactericidal qualities were lost by heating to 56° C. for 30 minutes: they could further state that an inactive antityphoid serum could be reactivated by means of fresh milk. Other authors also believe the bactericidal properties of milk to be due to antibodies of the same nature as those found in serum.

No doubt bacterial antibodies may pass from the serum of the mother into the milk. Bactericidal antibodies of different sorts have been found in milk. Pfaundler and Moro (1907) were able to demonstrate hæmolytic complement in milk. But some authors have found antibodies present in colostrum only. Pfaundler (1915) recapitulates that possibly "only colostrum contains antibodies, and even this only when present in sufficient quantity." Rosenau and McCoy (1908), Heinemann and Glenn (1908) were able to state that *B. typhosus* is agglutinated in milk, and they formed the opinion that this agglutination "bears some relation to the apparent decrease in the number of bacteria observed in fresh milk." Further, the leucocytes in milk have been found to possess phagocytic power. Rullmann and Trommsdorff (1906) mentioned a direct proportion between number of leucocytes contained and the bactericidal properties of milk. Rosenau and McCoy have directly observed this phagocytosis by the milk leucocytes, but these authors—as well as Moro (1907)—thought this of little consequence, because bactericidal properties could be demonstrated in milk tests where the leucocytes had been removed by centrifugation.

OXYDASE IN MILK.

Another factor may be brought in relation to the so-called bactericidal properties of milk, but has hitherto not been discussed in the literature on the subject. By means of its oxydases and peroxydases, milk is in possession of oxidizing power. The peroxydases and oxydases of milk generally are tested by means of Storch's reaction, which is accomplished in the following way: to 5 c.c. milk is added 1 drop of 0·2 per cent. solution of hydrogen peroxide and 1 drop of a 2 per cent. solution of paraphenylendiamin. If oxydases and peroxydases are present an indigo colour appears; if not, the milk remains unchanged. We have tested the milk after being heated for 15 minutes to 70° and 75°C. The milk heated to 70° always gave a positive Storch's reaction. The milk heated to 75° always remained unchanged. By means of this reaction, Storch found that the peroxydases are rendered inactive instantaneously at 80° C.; after 5 minutes at 75°C.; after 30 minutes at 72·5°C.; after 5 hours at 70°C. As stated above, we found bactericidal properties in milk heated to 70°C. for 15 minutes. But these properties could not be found in milk heated for 15 minutes at 75°.

We have thought it possible that these enzymes, on account of their oxidizing power, may bear some close relation to the bactericidal properties of milk. It is very striking that both the bactericidal properties of milk and the oxydases and peroxydases have got the same limits of temperature, being both rendered inactive after heating to 75°C. for 15 minutes.

The oxydases and peroxydases of milk are thought to be true products of

secretion, in contra-distinction to the catalases, which for the most part have been found to be produced by the bacteria of normal milk (Orla Jensen, 1907). The oxydases and peroxydases are further thought (Orla Jensen; Spolverini, 1904) to take their origin from the pasture, as oxidizing enzymes abound in this sort of food. In our ensilage it is likely that these enzymes may partly be altered.

We venture to suggest the theory that the bactericidal properties of milk bear some relation to the oxidizing enzymes of the milk, and we believe that the variability of the bactericidal properties of milk is caused by variations of the quantities of oxydases and peroxydases contained in different milk samples.

In the summer time when the cow was fed on pasture where oxidizing enzymes are said to abound, the milk was found in possession of well-marked bactericidal properties. In the winter time, when the cow was given ensilage, in which it is probable that these enzymes in part are destroyed by the fermentation, the bactericidal properties of the milk were less marked or quite lacking.

CONCLUSIONS.

(1) Fresh milk is found to possess bactericidal properties when tested with *B. typhosus* and *B. paratyphosus B*. This effect is demonstrable during the first 1–4 hours at 37° C. The decrease is evidently only temporary, and not traceable after 24 hours.

Kept at the temperature of the room a similar decrease of the bacteria takes place, starting later, but being of a longer duration.

(2) The bactericidal properties of milk have been found to vary greatly in milk samples from the same cow at different seasons of the year.

(3) These bactericidal properties are further found in milk heated to 63°C. for 30 minutes, and in milk heated to 70°C. for 15 minutes. This shows the fundamental difference between these bactericidal properties of the milk and the alexines of the serum, which are much less heat-resistant.

After heating to 75°C. for 15 minutes the bactericidal properties of the milk were lost.

(4) The theory is proposed that the bactericidal properties of milk are in close relation to the oxidizing enzymes of the milk.

(5) It is probable that the variations in the bactericidal power found at different seasons of the year, depend upon variations in the food of the cow, influencing the content of oxidizing enzymes in the milk.

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THE EFFECTS OF INCREASED INTRACRANIAL PRESSURES ON THE MEDULLARY CENTRES.

SHIRO TSUBURA
(Tokio).

From the Physiological Laboratory, St. Bartholomew's Hospital Medical College, London.

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THE experiments to be described in the present communication were made with the view to investigate the effects of alterations of intracranial pressure on the condition of the central nervous system.

It has been shown by Dixon and Halliburton (1914), to whose paper the reader is referred for the older literature on the subject, that a rise of intracranial pressure leads first to stimulation, and later to paralysis of the medullary centres; stimulation was least pronounced, and paralysis most evident, with the respiratory centre, which they found to be the first among the great bulbar centres to fail, whereas Cushing (1901) had found the respiratory centre to fail later than the vaso-motor centres. Dixon and Halliburton found the cardio-inhibitory centre to be the next to fail, and the vaso-motor centre the last. Recovery took place in the reverse order when the pressure was removed. The objects of the present investigation were to re-investigate the whole question, and particularly to examine the order of failure of the bulbar centres, since the work of Dixon and Halliburton was carried out on dogs anæsthetized with morphine and urethane, which might have been held to be responsible for the early failure of the respiratory centre.

In view of the different susceptibilities of the cells of different nerve centres to oxygen lack (*cf.* Cannon, 1923), pronounced differences of behaviour were certainly to be expected. It was also desirable to ascertain whether, under the altered conditions brought about by rise of intracranial pressure, there was any change in the reflex excitability of the nervous centres.

Methods.—With one exception, in which a dog was used, the experiments were made on cats; there seemed to be very little difference attributable to difference of species. As anæsthetics, paraldehyde, light ether or urethane were used; identical results were obtained with all. In the majority of the experiments the following procedure was adopted for altering the intracranial pressure: A trephine hole 1·3 cm. in diameter was made at the most prominent part of the parietal bone on the right side, and bleeding quenched by rubbing plasticine into the cut edge of the diploë; into the hole a rubber bung, bearing a glass tube flush with its inner surface, was inserted. The glass tube was led through a T-tube to a bottle filled with warm gum-saline solution; the gum-saline bottle was provided with a 3-hole stopper; through one hole passed the tube leading from the bottom of the bottle to the trephine-hole tube just described; through the second hole a tube passing just below the stopper led to a Hürthle manometer (for recording the pressure), and a branch from this led off to a mercury manometer for reading off the pressure; the tube in the third hole also

passed just below the stopper and led, *viâ* a 3-way tap, to a large bottle filled with air and provided with a Hg. manometer and cycle pump, with which the air-pressure could be increased. When the intracranial pressure was to be raised, a little of the warm saline solution was first run into the trephine tube and out again by the side-tube of its T-connection, to ensure uniformity of temperature; then, with the 3-way tap closed, the pressure was raised to the desired extent in the air-reservoir, and finally, at the required moment, the air-pressure was transmitted to the gum-saline solution by turning the tap. The large size of the air-reservoir ensured constancy of intracranial pressure for long periods. If it was desired to raise the intracranial pressure slowly and uniformly, it was only necessary to interpose between the air- and saline-reservoirs a suitable length of fine glass capillary (piece of thermometer tubing). There was, however, no conspicuous difference between the effects of sudden and of slow increase of pressure.

Some of the experiments were carried out on decerebrated animals. Decerebration was performed under deep $CHCl_3$ anæsthesia by severing the brain-stem with a blunt instrument passed through the trephine hole, thus effecting transection, sometimes rather obliquely, at the level of the anterior colliculi, the guide being the anterior edge of the bony tentorium. After the operation the trephine hole was not closed until bleeding had ceased. Decerebration by the injection of starch by Langley's method was tried on three occasions, but was found to give inconstant results.

Arterial pressure was recorded from the right carotid artery; as pointed out by Roberts (1921), the effect of tying off one carotid is negligible; respiration was recorded by means of a tambour connected with the side-branch of the trachea-tube.

In a few of the experiments, instead of trephining the calvarium, a trocar and cannula was passed into the sub-cerebellar cisterna in the fashion described by Dixon and Halliburton; the intracranial pressure was raised by connecting this cannula with the pressure apparatus described above. This method, however, is much more difficult to carry out in cats than in dogs on account of the risk of mechanical damage to the medulla which attends it. In other experiments a small trephine hole (6 mm.) was made in the occipital bone, the dura opened beneath, and a tube leading to the pressure apparatus inserted.

GENERAL EFFECTS OF RISE OF INTRACRANIAL PRESSURE.

Experiment showed that the distribution of the pressure change brought about by applying pressure by the three different methods was not quite the same. When the pressure was applied direct to the cerebrospinal cavity it was presumably equally distributed throughout the central nervous system, including its blood-vessels. When applied outside the dura, on the other hand, the pressure in the cerebrospinal cavities did not show a parallel alteration; when the epidural pressure was raised to 160 mm., for instance, that in the cerebrospinal cavities had slowly risen to 25 mm. Hg., and with further increase of epidural pressure showed no further increase, even when it was as high as 220 mm. Hg. It would seem that under these circumstances the pressure applied outside the dura is more or less localized owing to fixation of the dura, while the brain substance just beneath the application of the pressure is greatly compressed and transmits its pressure unequally, though especially towards the base of the skull, where the large blood-vessels are compressed against the bony wall. But since the application of pressure outside the dura affects not only the brain but also the spinal cord, it is evident that the effects are widely, if not equally, distributed through the entire nervous system.

Whichever method of raising the intracranial pressure was adopted, and whether the dura had been incised or left intact, the results were essentially the same, though it was of course sometimes difficult to compare the results of different methods of compression on the same animal because of the rapid deterioration of the bulbar centres as a result of compression.

The question has often been asked (Bayliss, 1923) whether the medullary

centres are directly sensitive to pressure. Franck (1877) has, indeed, given some evidence that they are. I have made no direct experiments which enable me to give any definite answer to this question, but there does not seem to be much indication that pressure *per se* plays at all so large a part in producing the effects as does the anæmia set up by it. Thus the effects of the anæmia resulting from rise of intracranial pressure are essentially similar to those accompanied by a fall of pressure, such as the influence of gravity (L. Hill, 1895), hæmorrhage (Bayliss, 1920; Barry, 1923), or ligation of vessels (Stewart, Guthrie, Burns and Pike, 1907).

A much more likely factor than mere pressure in the present experiments is an actual displacement of the nervous structures owing to the inequality of pressures already referred to. Slight movement of the cerebellum can in fact be observed through an occipital window when pressure is applied in the parietal region. A definite dripping of fluid from the nose, which we witnessed towards the end of one experiment during application of high extra-dural pressures, is further evidence of displacement of tissues by repeated pressure changes.

The cerebral cortex is very easily paralysed by pressure, and it has long been known that profound anæsthesia follows when a foreign body is introduced into the skull through a trephine hole (Dalton, 1876; Howell, Budgett and

Experiment 1: *Cat. Light ether. Extra-dural Pressure applied in Parietal Region.*

Time.	Extra-dural Pressure.	Pupils.	Conjunctival reflex.	Knee-jerk.	Movements.
11.52	32 mm. Hg.	½ dil.	Brisk	—	Some
11.55	50 „	„	„	—	„
12.8	95 „	„	„	Brisk	„
12.11	108 „	¼ dil.	Dull	„	—
12.14	140 „	¾ „	None	None	None
12.17	60 „	—	—	—	—
12.19	60 „	½ dil.	Dull	—	—
12.24	130 „	—	—	—	—
12.25	130 „	Full dil.	None	None	Rigidity
12.28	130 „	„	„	Slight	Flaccid
12.29½	45 „	—	—	—	—
12.35	45 „	—	Sluggish	—	Rigid
12.36	7 „	½ dil. (react to light)	Very brisk	—	„
12.38	Gave more ether	—	—	—	—
12.40	7 mm. Hg.	¼ dil. (L. wider)	Good	Good	—

Up to 100 mm. Hg. the effect of the pressure was very slight; thereafter there is some constriction, followed by dilatation of the pupil and loss of conjunctival reflex. The knee-jerks are also abolished when the intracranial pressure reaches 140 mm., and recover slowly later, when the pressure is reduced. Compression and decompression are often, as in this experiment, accompanied by rigidity of the skeletal muscles, with a posture resembling that seen in decerebate rigidity.

Leonard, 1893), just as it does in cases of acute cerebral compression in man. It was therefore not surprising to find that during the application of extra-dural pressure, under light ether anæsthesia, the conjunctival and other reflexes speedily vanished, as shown in the protocol on p. 283.

EFFECTS ON THE RESPIRATORY CENTRE.

Apart from minor differences due to differences in pressures, to times of application, rapidity of rise of pressure, etc., it may be said that in general, the effect of application of extra-dural pressures below 100 mm. Hg. is but slight, and sometimes causes slight slowing, more often slight acceleration of rate of respiration. Pressures from 100–150 mm. cause at first considerable increase both in rate and in depth, but this is soon followed by great retardation, or by stoppage. When the pressure is over 150 mm., and is abruptly applied, the phase of hyperpnœa is very brief, and is abruptly followed by complete stoppage (Fig. 1), similar to that following ligature of all the vessels (Roberts, 1921). Though these are the usual effects, they are liable to modification as a result of contemporary changes in the circulation. Thus, on raising the intracranial pressure, the breathing may be at first amplified, but afterwards fall back, with rising blood-pressure, to the normal; again, as shown in Fig. 2, there may be a sudden short panting respiration instead of a stoppage, while at higher pressures, if there has been a good vaso-motor response, the respiration may be slowed instead of being suspended.

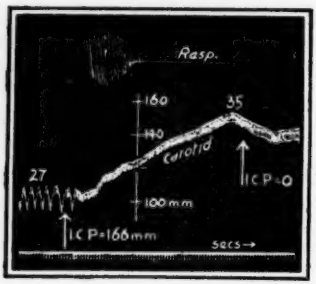

FIG. 1.—Suprarenectomized cat under paraldehyde. Intracranial pressure is changed at the arrows. The response is the same as in intact animals.

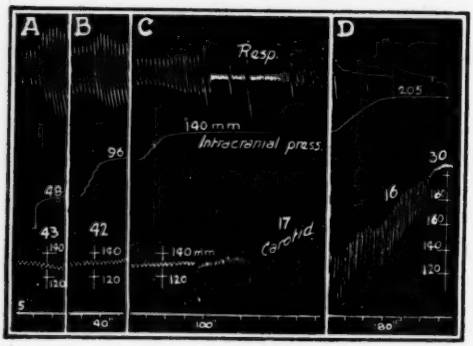

FIG. 2.—Cat under paraldehyde. Shows effect on respiration and pulse of slowly raising the intracranial pressure. Figures under the time tracing indicate seconds from the commencement of the experiment; those above the carotid tracing indicate pulse-beats per 10 secs.

But in more than 30 experiments there has been no evidence contrary to that of Dixon and Halliburton that the respiratory centres fail long before the vaso-motor centre (Fig. 1), and, like them, one thinks that the respiratory centre is by far the most sensitive to the effects of raised intracranial pressure. After the pressure has been lowered again (with artificial respiration in the meantime, if necessary) the respiratory centre recovers only slowly from the effects; often it shows no recovery. During the recovery (but not usually during the actual compression) various types of periodic breathing are

encountered (Fig. 3, A), and the first recovered breaths are invariably of a gasping character (Fig. 3, B). These effects are no doubt explicable in the light of Lumsden's (1923) experiments as due to different effects on the various respiratory centres, the lower centre being the first to recover, while the apneustic and pneumotaxic centres recover more slowly, if at all.

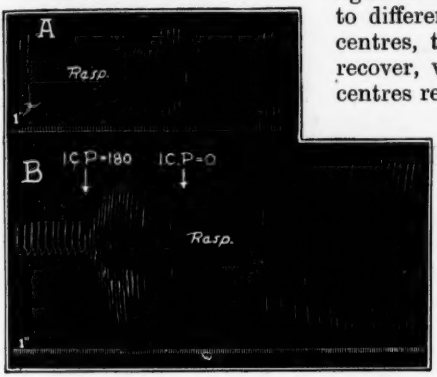

FIG. 3.—Cat under paraldehyde. Two types of respiratory recovery after arrest by high intracranial pressure. In "A" the pressure was reduced to 0 mm. Hg. before the commencement of the portion of tracing shown.

EFFECTS ON VASO-MOTOR CENTRES.

The effects of rise of intracranial pressure on the vaso-motor centres have been frequently described, and we have had ample opportunity of confirming the account given by previous workers of the blood-pressure rise which tends to overcome the bulbar anæmia. The main cause of the rise of blood-pressure is evidently stimulation of the vaso-constrictor centre; cardiometer records showed that there was no increased cardiac output to account for it. That the effect of the intracranial pressure is on the medulla and not on any higher or lower centres is shown by two experiments: first, the rise of blood-pressure is seen in characteristic fashion in a decerebrated animal (Fig. 4); secondly, after destruction of the medulla by injection of starch grains into the cranial ends of both carotid arteries, or after division of the spinal cord in the cervical region, alterations of pressure in the cranial cavity produce absolutely no corresponding changes in the arterial pressure. The spinal vaso-motor centres seem to be quite unaffected by such pressure, though Dale and Lovatt Evans (1922) have shown that they are by no means insensitive to changes of carbon dioxide content of the blood, and also in spite of the fact that spinal reflexes are by no means unaffected when the intracranial pressure is raised.

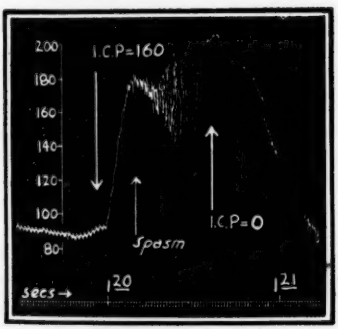

FIG. 4.—Decerebrate cat under paraldehyde. Here also a distinct rise in blood-pressure follows increase in intracranial pressure.

In slight or early stages of compression there may be some vagal cardiac inhibition, though this is generally less marked in the cat than it is in the dog.

The blood-pressure response is almost unaffected by section of both splanchnics, by removal of both suprarenals, or by both procedures combined (Fig. 5). It is therefore clear that the splanchnic area is not of such importance

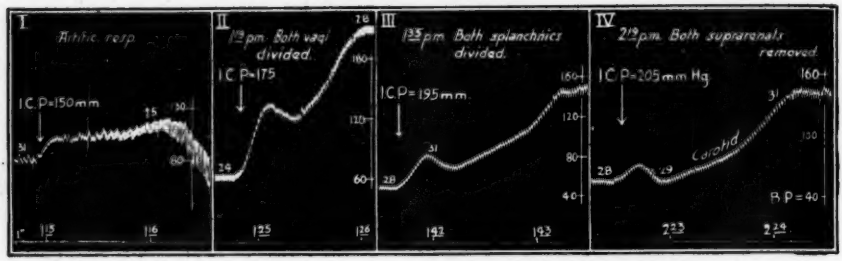

FIG. 5.—Cat under paraldehyde. Artificial respiration. Blood-pressure response to increased intracranial pressure is not materially affected by (*a*) section of vagi, (*b*) section of splanchnics as well as vagi, (*c*) section of vagi and splanchnics, and removal of suprarenals.

as might have been supposed. The same fact is illustrated by an experiment on an eviscerated cat, in which the arterial pressure rose ultimately from 110 mm. to over 200 mm. Hg. when the intracranial pressure had been kept at 162 mm. for 2 minutes (Fig. 6). Since removal of the suprarenals makes very little difference to the response, it is also certain that stimulation of the chromaffin tissue, though it may occur, plays no important *rôle* in causing or maintaining the high blood-pressure.

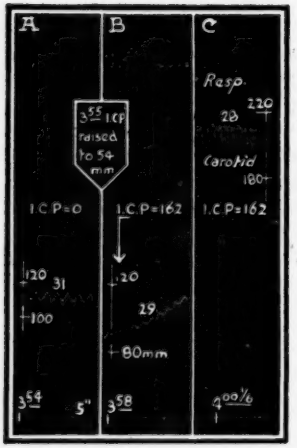

FIG. 6.—Cat under paraldehyde. Stomach, intestine and spleen removed. While intracranial pressure is kept constant at 162 mm. Hg. there is a considerable rise in blood-pressure.

In a few cases the application of small pressures led to a definite fall of blood-pressure; this is illustrated by Fig. 7 and by Figs. 2, A, and 6, there being in neither instance any cardiac inhibition to account for the fall. But the fall has never been seen when both vagi have been divided, which suggests that it may be due to an exaggerated excitability to normal reflex stimulation of the depressor centre, or of some parts of the reflex arc (Scott and Roberts, 1923), in consequence of the altered conditions of circulation.

The condition of excitability of the various centres for vascular control was tested at different pressures by stimulation of the central end of the divided median, anterior crural or sciatic nerves, in the examination of pressor reflexes, and of the central end of the vagus, after division of both vagi, for the depressor reflexes. At intracranial pressures below 100 or 150 mm. pressor responses were either unaltered, or even slightly reduced. At rather higher pressures, when a definite rise of blood-pressure had occurred, there was if anything an increased excitability, for, as shown by Fig. 8, even when the arterial pressure had risen to 220 mm., it was still possible further to increase it by 25 mm. by

stimulation of the central end of the median nerve; the centre, therefore, though excited by the anæmic conditions, is still capable of showing a further reflex increase, which led to a degree of vaso-constriction which must have been almost maximal.

Very high pressures produced paralysis of the vaso-motor centre, and led to a fall of blood-pressure; usually more than 200 mm. was necessary for this.

The vaso-dilator reflexes exhibited a different behaviour; at moderate pressures there was usually no reduction of the depressor reflex; sometimes it was even slightly increased, even when there was no rise of blood-pressure (Figs. 9 and 12). But this state of affairs only held good for moderate pressures or for a short time. At higher pressures, say of 200 mm. or over, or after long or repeated application of smaller pressures, the vaso-dilator reflexes were lost altogether, or even replaced by pressor responses. Sometimes this replacement by pressor reflexes was evident in spite of there having been one intact vagus, so that the entire depressor mechanism showed a reversal—a fact of some physiological significance (Figs. 9 and 10).

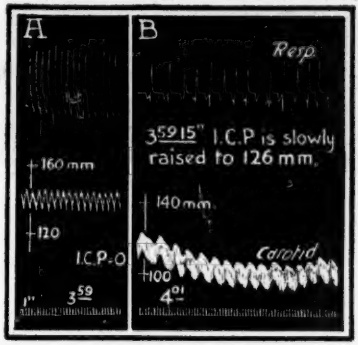

FIG. 7.—Cat under paraldehyde. Fall in arterial pressure accompanying *small* increase in intracranial pressure.

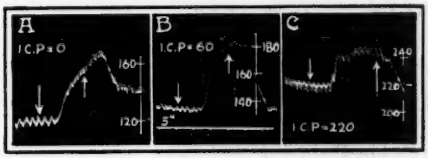

FIG. 8.—Cat under urethane. Left vagus divided. Between the arrows, stimulation of central end of divided left median nerve. Coil at 10 cm. The higher the intracranial pressure the greater the rise in blood-pressure.

Since ergotoxin paralyses the motor functions of the sympathetic (Dale, 1906), it might have been expected that any effect of raised intracranial pressure on the vaso-dilator mechanism might have been revealed after administration of ergotoxin. This was not the fact, however, for after injection of Femergin, rise of intracranial pressure produced no effects whatever. It seems that not only the peripheral motor

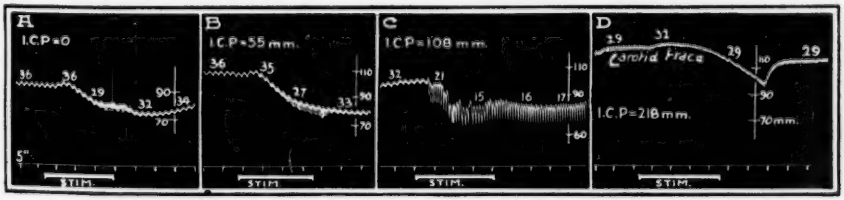

FIG. 9.—Cat under paraldehyde. Artificial respiration. Stimulation of central end of vagus. Coil at 8 cm. Fall in blood-pressure is increased at moderate, but reduced at higher intracranial pressures.

sympathetic mechanism but the synaptic bulbar structures were put out of action by this drug—a view confirmed by the observation that vascular reflexes could now no longer be elicited, although injection of adrenalin gave the expected fall of blood-pressure.

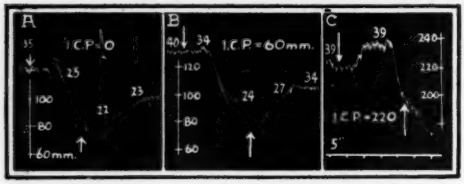

FIG. 10.—Cat under urethane. Stimulation of central end of left vagus between the arrows. Coil at 10 cm. Fall in blood-pressure follows stimulation at moderate intracranial pressures, but a rise follows at high pressures.

EFFECTS ON THE HEART-RATE.

In the dog, the vagus centre, as is well known, is easily excitable, and the effect of a sudden considerable rise of intracranial pressure may be so pronounced as to resemble that of peripheral vagus stimulation (Fig. 11), the blood-pressure in consequence showing at first a well-marked fall.

In the cat, the effects of the pressure do not seem capable of reproduction with the same precision as in the dog, because the effects on the less excitable inhibitory mechanism are often outweighed by other contemporary effects, such as an outpouring of adrenalin from chromaffin tissue, an effect on the cardio-accelerator centre, and paralysis of the cardio-inhibitory centre. Nevertheless the most conspicuous of these effects is usually the primary excitation of the vagus centre, and its subsequent failure at higher pressures; in the cat, definite cardiac inhibition, which is abolished by section of both vagi, is usually seen at extra-dural pressures of 150–200 mm. Hg. (Fig. 5). When the vagi have been divided, or even sometimes when they are intact, a slight acceleration of the heart may occur at moderate intracranial pressures such as 150–200 mm. Hg. This acceleration seems to be chiefly due to liberation of adrenalin into the circulation, since it is nearly abolished by removal of both suprarenals. But it is not entirely abolished by this operation, as the following experiment shows:

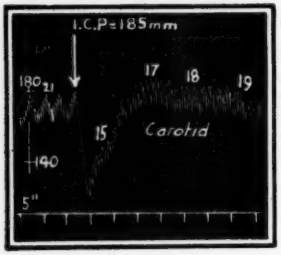

FIG. 11.—Dog under morphine and urethane. Raising of intracranial pressure is followed by changes in the pulse similar to those which normally follow vagus stimulation.

Exp. 2: *Cat* (♂). *Paraldehyde. Artificial respiration.*

	Pulse-rate per 10″— When intracranial pressure =0.	When intracranial pressure =200 mm.
Vagi and suprarenals intact	29	27, 26, 30
Suprarenals tied off	36, 34	24, 34
Section of vagi	37, 37	42

In Fig. 12, also, comparison of B (i) and B (iv) before stimulation of vagi shows also an increase of 2 beats per 10″ on raising the intracranial pressure after section of vagi and removal of both suprarenals. Whether this small residual acceleration may be attributed to an effect on the cardio-accelerator centre—a possibility suggested by Tigerstedt (1893)—or whether it represents some chemical mechanism, such as that studied by Cannon and his associates (1922), or a formation of adrenalin from accessory chromaffin tissue like that suggested by Kellaway (1919), I have not attempted to decide.

The excitability of the cardio-inhibitory centre to reflex stimulation was studied by excitation of the central end of the vagus.

In some instances the centre showed no appreciable change until high pressures had been reached, when suddenly its excitability disappeared. In other instances, at pressures which produced little effect on the tonic activity of the centre, there was a definite increase of excitability (Fig. 9), and there

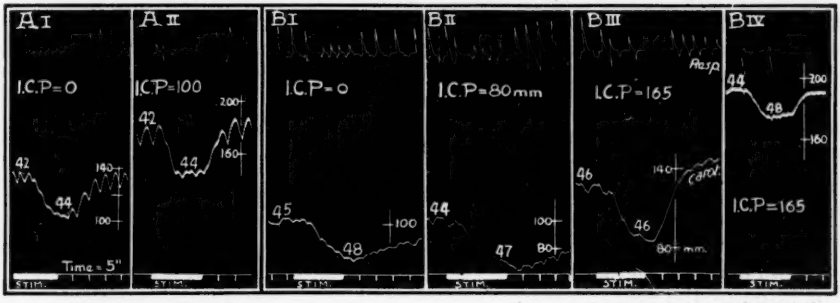

FIG. 12.—Cat under paraldehyde. Coil at 15 cm. Stimulation of central end of left vagus. In A i and A ii both vagi divided; in B i, ii, iii and iv both suprarenals removed also. B iii is at 1 minute after the raising of intracranial pressure to 162 mm. Hg. B iv is at 5 minutes after the rise and shows a smaller response to stimulation than does B iii.

seems to be no doubt that certain grades of anæmia produced by compression —unlike that produced by hæmorrhage in dogs (Barry, 1923), brings about not only an increase of tonic activity, but also an augmented excitability of the centre to reflex stimulation.

At higher pressures the cardio-inhibitory centre undergoes sudden paralysis, shown by an abrupt acceleration of pulse-rate (Fig. 2), and further improvement in the arterial pressure. On reflex excitation at this stage no inhibition results (Fig. 10), but there may be a slight acceleration instead. When the pressure is lowered again, the vagus tone, and with it the reflex excitability of the centre, returns slowly, but not without showing traces of impairment if the pressure has been kept up for long.

THE PART PLAYED BY THE SUPRARENALS.

These experiments have shown that the suprarenals play only a small part in causing the rise of blood-pressure in response to anæmia of the nerve centres.

Since it has been claimed by Roger (1924), however, that the activity of the suprarenals is the principal factor responsible for the maintenance of the arterial pressure in cerebral embolism, and that this high pressure is not kept up when cerebral embolism is produced in a decapsulated animal, some attempts were made to repeat on cats the experiments which he had made principally on rabbits. Embolism was produced by the injection of starch grains according to Langley's (1912) method. It so happened that the embolism which resulted gave, on cats with *intact* suprarenals, results similar to those obtained by Roger with decapsulated rabbits. It follows, therefore, that there is either some great difference between these species, or else—and more probably—that the fortuitous site of the embolism accounts for the apparent difference which he saw between intact and decapsulated rabbits. In my experience it is impossible to forecast whether, on producing an embolism, the circulation through the vaso-motor centres will be completely stopped or merely restricted; yet in the former case, the blood-pressure would not be sustained, while in the latter it might well be. In any case, I can find no statement in Roger's work to the effect that the rise of pressure caused by embolism is held in abeyance when the suprarenal veins are clipped, to reappear when they are released. In my opinion, it has so happened that in Roger's decapsulated rabbits there was a more extensive embolism than in the intact ones.

DISCUSSION.

The results in general confirm those obtained by Dixon and Halliburton. It has also been possible to carry the analysis of the effects somewhat further than has been done by previous observers.

The effects obtained are undoubtedly due for the most part to the anæmic condition of the central nervous system which results from the application of pressure to structures enclosed in bony walls. But it does not necessarily follow that all procedures which produce anæmia will necessarily give the same effects. When the intracranial pressure is raised, we are really producing at least two different conditions—quite apart from any possible effects due to direct effects of pressure or to mechanical displacement of tissues—namely, a reduction of the normal gaseous exchanges of the nerve tissues, and also a reduced rate of blood-flow through them. It is not surprising, therefore, that the results differ in some minor respects from those obtained by other methods which are accompanied by different circulatory conditions, such as asphyxia (Mathison) or hæmorrhage combined with blood dilution (Barry). The effects of generalized oxygen deficit, as in the conditions named above, or in the related effects of cyanide poisoning (Lovatt Evans, 1919), are also necessarily different from those of localized anæmia of the nerve-centres, so it is not surprising that such peripheral effects as heart-block or vascular paralysis are wanting in the phenomena under discussion.

As regards the effects on the various centres, it seems certain that, of the great medullary centres, the respiratory centre is the earliest and the most profoundly affected by rise of intracranial pressure. Death from failure of respiration would, therefore, appear to be the most imminent danger in cases where the intracranial pressure is suddenly raised (depressed fracture, etc.).

This centre is also the last to recover its function when the intracranial pressure has been restored to normal after a period of compression, and suffers most acutely from after-effects, which take the form of periodic respiration of various types. The centre which is next to be affected is the cardio-inhibitory centre, which shows early stimulation, followed by paralysis. Stewart, Guthrie, Burns and Pike, in experimental anæmia by ligature of the vessels, found that the respiratory and cardio-inhibitory centres failed at about the same time, but I have always found the respiratory centre to fail the earlier. These experiments, considered in the light of researches on experimental hæmorrhage, and the work of Mathison (1911) on the effects of asphyxia on the bulbar centres, suggest that the stagnation of the blood is a factor at least equal in importance to the deprivation of oxygen. Anoxæmia of the centres produced by hæmorrhage or by asphyxia leads to a much greater display of hyperpnœa than was seen in the present series of experiments; further, the effect of hæmorrhage and blood-dilution on the cardio-inhibitory centres seems to be quite different from that of intracranial compression, the former reducing inhibitory tone (Barry) and reflex excitability, the latter increasing them. It is true that the vagus centre is ultimately paralysed by very high intracranial pressures, but it seems very unlikely that the degree of oxygen-lack in experimental hæmorrhage is of so high an order. An accumulation of waste products in the latter case is the probable explanation of the difference; it has been shown by Bainbridge (1920) that the cardio-inhibitory centre is stimulated by a fall in the pH of the blood, such as would occur when there was an accumulation of acid metabolites.

The vaso-motor centre comes after the cardio-inhibitory centre in order of sensitiveness to raised pressure. In the anæmia of hæmorrhage the vaso-constrictor centre is also stimulated, as shown by Bayliss (1916). The effect of raised intracranial pressure on the centre is powerful and lasting, and when there is recovery of centres paralysed by high pressure, after release of the pressure it is the first one to regain its tone. Comparison with the hæmorrhage experiments again suggests that there is some difference between the two effects, for Barry found that the vaso-motor centre was the first of the three great centres to show permanent damage (in dogs). Bayliss (1920) certainly found, as I have done here, that the respiratory centre failed before the vaso-constrictor centre.

SUMMARY.

(1) Raised intracranial pressure acts first on the respiratory centre, causing transitory hyperpnœa, followed by sudden stoppage of respiration. The findings agree with those of Dixon and Halliburton.

(2) The centres next to be affected are the cardio-inhibitory and vaso-constrictor centres, in the form of an increase in the tonus and in reflex excitability. There are indications also of alterations in the cardio-accelerator and vaso-dilator centres. The vaso-constriction affects the whole body.

(3) There is some liberation of adrenalin, but this does not represent an important factor in causing the rise of blood-pressure.

(4) High pressures lead to paralysis of the cardio-inhibitory, and finally of the vaso-constrictor centres.

(5) The effects produced are partly the result of anæmia, and partly due to the diminution of blood-flow, with attendant accumulation of waste products.

It gives me pleasure to express my thanks to Prof. C. Lovatt Evans for his assistance and advice during the course of this work.

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THE CHOLESTEROL OF BLOOD IN MALIGNANT DISEASE.

ARCHIBALD NEIL CURRIE, M.A., B.Sc., A.I.C., F.C.S.

From the Research Department of the Glasgow Royal Cancer Hospital.

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THE literature on the pathological occurrence and distribution of cholesterol is voluminous, and this substance has by several investigators been assigned an important *rôle* in the ætiology of cancer.

Robertson and Burnett (1913) have attempted to show that the blood and tissue concentrations are closely related with the rate of cancer growth—lecithin acting as a growth inhibitant, cholesterol as a stimulant. Webb (1901) treated cases of inoperable cancer on the assumption that loss of cholesterol from the normal cell was a determining factor in the cell proliferation of cancer. Bullock and Cramer (1914), in their investigations on mouse cancer, have found that the cholesterol concentration is inversely proportional to the rate of growth.

The results obtained in the blood determinations of cholesterol are equally conflicting. Luden (1916) and De Niord (1920) report a pronounced hypercholesterolæmia; while Denis (1917) and Wacker and Hueck (1913), on the other hand, have found low values in diseases characterized by cachexia and prostration.

These varying results and their superimposed interpretation are not surprising when one considers that there are at least five different "normal" blood cholesterol values given in the literature. Hoppé-Seyler gives the normal blood-content of cholesterol as 0·25 per cent.; Bloor as 0·22 per cent.; Grigaut as 0·09 per cent.; Gorham and Myers, as 0·16 to 0·17 per cent. In view of these figures it is difficult to determine whether a condition either of hypercholesterolæmia or hypocholesterolæmia exists in any given subject.

EXPERIMENTAL.

Blood-plasma and occasionally corpuscular cholesterol determinations were carried out in this laboratory over a period of twelve months. Blood-plasma rather than blood-serum was chosen, as it has been shown recently by Jung and Wolff (1922) that the spontaneous coagulation of blood is always accompanied by an accession of lipin substances, including cholesterol, to the serum. In all the cases examined, cholesterol was equally distributed between the plasma and the corpuscles.

Grigaut recommends a special technique for the determination of cholesterol, but in our experience an ordinary burette, several graduated 15 c.c. cylinders, an evaporating basin, a centrifuge tube and a microburner comprised all the apparatus required for accurate analysis.

For our purpose, the oxalated blood was centrifuged in a high-speed centrifuge, and the plasma was rapidly transferred to a small measuring

TABLE I.—*November to January.*

Case.	Age.	Nature and rate of growth.	Percentage cholesterol.
1	57	Carcinoma of tongue, ×××	0·070
2	52	Pigmented sarcoma, ××	0·070
3	62	Carcinoma of tongue, ×××	0·078
4	55	Elephantiasis, scrotal, ×	0·076
5	57	Sarcoma of orbit, × (40 years' duration)	0·075
6	59	Epithelioma of floor of mouth, ×××	0·071
7	54	„ of pharynx, ×××	0·078
8	65	„ of lip, ××	0·090
9	54	Sarcoma of lower jaw, ×××	0·073
10	52	„ „ ×××	0·081
11	53	Carcinoma of thyroid, ×	0·070
12	38	Cancer of breast, ×	0·070
13	66	Rodent ulcer of nose, ×	0·100
14	68	Carcinoma of bowel, ××	0·078
15	59	„ of tongue, ××	0·070
16	65	Epithelioma of neck, ×××	0·090
(17)	69	Sarcoma of lower jaw, ×	0·380 !

Average pathological value . . . . 0·077 %.
Average normal value . . . . . 0·110 %.

NOTE.—The numbers in the first columns of these tables indicate subjects. Where a number is repeated it indicates that a second or third determination of cholesterol content has been made on the same individual at the period recorded in the protocol. The cholesterol percentages are tabulated according to the chronological order of determination.

× = Slow-growing. ×× = Moderate. ××× = Rapid-growing.

cylinder, 1·5 to 2·0 c.c. being taken. The plasma was shaken up with 15 c.c. 60 per cent. alcohol to which had been added 5 c.c. 0·2 N. NaOH. The solution was then transferred to a 50 c.c. burette, and the measuring cylinder was carefully washed out with three instalments of 5 c.c. ether. The ether washings were added to the burette, which was twice inverted and allowed to stand. After equilibrium was established in the two-layer system, the bottom layer was almost completely drawn off, and the superincumbent ether layer was washed with 20 c.c. distilled water. The aqueous layer was completely drawn off, and the cholesterol-containing ethereal layer was transferred to an evaporating basin. The burette was finally washed with 5 c.c. ether, which

was drawn off into the evaporating basin. The basin was now placed in the hot water-bath to expel ether, and the residual cholesterol was finally heated gently to dryness over the micro-burner.

This residue was taken up with 5 c.c. chloroform in a small measuring cylinder, and estimated colorimetrically in the ordinary way. The standard was a 0·06 per cent. chloroform solution of cholesterol, and the colour was

TABLE II.—*February to April.*

Case.	Age.	Nature and rate of growth.	Percentage cholesterol.
5	57	Sarcoma of orbit, ×	0·171
18	70	Carcinoma of bowel, ××	0·097
19	48	Sarcoma of jaw, ××	0·119
20	41	Lymphadenoma, ×	0·076
21	60	Sarcoma of lower jaw, ×××	0·130
22	60	,, ,, ×××	0·130
23	69	Epithelioma of floor of mouth, ××	0·119
24	49	,, of tongue, ×××	0·095
15	59	,, ,, ××	0·098
2	52	Pigmented sarcoma, ××	0·130
25	45	Epithelioma of lower lip, ×	0·270
26	56	Carcinoma of tongue, ××	0·106
27	26	Epithelioma of left cheek, ×××	0·102
28	53	Carcinoma of larynx, ×××	0·081
29	67	,, of tongue, ×	0·130
30	46	Rodent ulcer of lower eyelid, ×	0·175
31	74	Epithelioma of floor of mouth, ×××	0·120
32	77	,, of tongue, ××	0·210
15	59	,, ,, ××	0·180
5	57	Sarcoma of orbit, ×	0·280
33	73	Cancer of œsophagus, ×	0·150
34	68	,, of rectum, ×	0·260
35	44	Sarcoma of forearm, ×	0·160

Average pathological value . . . . 0·14 %.
Average normal value . . . . . 0·18 %.

developed by the addition of 2 c.c. of acetic anhydride, and 4 drops of 66 per cent. H_2SO_4.

The standard and unknown solutions were compared when each gave a definite absorption band in the red end of the spectrum (between λ 6700 and λ 7000). Well-defined selective absorption in the red was invariably manifested in half an hour. The identity of the absorption band in each case was taken as a criterion of the absence of such so-called oxidation products as isocholesterol in the blood, since the time-rate of development of equal widths of band for equal concentrations of known and unknown was the same.

RESULTS.

In Tables I, II, III, IV are given the blood-cholesterol values for over seventy cases determined over a period of a year. The results are tabulated according to season: Table I gives values for the period November to January; Table II, February to April; Table III, May to July; Table IV, August to October. The average normal blood-cholesterol value for each period, which was deduced from several cases evaluated here, is inserted at the foot of each table, along with the corresponding pathological average.

TABLE III.—*May to July.*

Case.	Age.	Nature and rate of growth.	Percentage cholesterol.
5	57	Sarcoma of orbit, ×	0·280
20	41	Lymphadenoma, ×	0·250
36	52	Sarcoma of jaw, ××	0·120
37	57	Cancer of tongue, ×××	0·150
38	62	„ „ ×	0·290
39	73	„ of œsophagus, ×	0·160
40	63	„ of cervical glands, ×	0·150
41	51	Epithelioma of floor of mouth, ×	0·240
42	69	Cancer of fauces and tongue, ×	0·210
43	61	Epithelioma of floor of mouth, ×	0·300
44	69	Cancer of tongue, ×	0·150
45	69	Epithelioma of chin, ×	0·200
46	16	Sarcoma of ilium, ×	0·290
47	75	Cancer of tongue, ×	0·210
48	52	„ of stomach, ×××	0·250
49	61	„ of throat, ×	0·240
50	48	„ of thyroid, ××	0·240
51	55	Epithelioma of external ear, ×	0·290
52	65	Rodent ulcer of nose, ×	0·240
53	51	Epithelioma of chin, ×	0·220
54	64	Sarcoma of neck, ×	0·220
55	69	Cancer of tongue, ××	0·150

Average pathological value . . . . 0·22 %.
Average normal value . . . . . 0·28 %.

DISCUSSION.

The figures given in the protocols give a partial explanation of the divergent cholesterol values obtained for normal blood by Bloor, Grigaut, Hoppé-Seyler and Gorham and Myers. These investigators overlooked the possibility of a seasonal variation in the amount of circulating cholesterol. In

this connection, it is interesting to note that a variation of a different type has been found by Kipp (1920), in his studies on the cholesterol content of serum in pneumonia. He finds a primary hypocholesterolæmia, the degree of which is dependent on the intensity of the infection, followed by a secondary hypercholesterolæmia during the period of convalescence, and continuing for a varying period of time after the resolution of the pneumonic exudate. Finally, there is a return to the normal cholesterol content of serum.

In cancer, however, which may be regarded as ætiologically abacterial,

TABLE IV.—*August to October.*

Case.	Age.	Nature and rate of growth.	Percentage cholesterol.
56	65	Rodent ulcer of nose, ×	0·150
57	71	Epithelioma of cheek, ××	0·130
58	64	Sarcoma of jaw, ××	0·120
59	53	Epithelioma of left side of neck, ××	0·170
60	63	,, of ear, ×	0·130
61	49	,, of floor of mouth, ××	0·170
5	57	Sarcoma of orbit, ×	0·140
62	37	,, of jaw, ×××	0·130
63	50	Cancer of colon, ××	0·180
64	49	Epithelioma of neck, ×	0·230
50	61	Cancer of throat, ××	0·110
53	51	Epithelioma of chin, ×	0·140
65	25	Carcinoma of rectum, ×××	0·140
54	64	Sarcoma of neck, ×	0·130
66	62	Epithelioma of hand, ×	0·130
67	55	Cancer of breast, ×	0·210
55	69	Carcinoma of tongue, ××	0·140

Average pathological value . . . . . 0·15 %.
Average normal value . . . . . 0·18 %.

there is no question of a serological adaptability of cholesterol, and we must look in another direction for an explanation of its changing concentration. In any assessment of its pathological significance, there are many complicating factors which have to be taken into account. One has to consider its origin, mode of transport and function, its chemical constitution, reactivity and relationship with other steroloid substances, its ultimate metabolic destiny, and the controlling factors of locality, nature of disease, its duration, growth-rate, age of subject, qualitative and quantitative nature of food ingested, and certain other physical factors.

The origin of cholesterol is still unknown. Some investigators would locate its genesis in the ingested plant sterols, such as phytosterol, which may undergo an intra-molecular change in the body. Krukenberg and Cotte ascribe

to it a carotenogenetic origin. Recently we have conducted a short investigation here on the influence of incubation on the cholesterol content of the hen's egg (occasionally exposed to strong sunlight), and also on the influence of ultra-violet radiation on the cholesterol content of rabbit blood. Our findings, which will later be the basis of a short communication, indicate that animal protoplasm is able to synthetize cholesterol, and that its progressive increase in concentration in tissue is paralleled by a corresponding decrease in carotene content (hen's egg). This may not mean, however, that cholesterol is able to build part of its molecule at the expense of carotene, as the changes which we have associated may be incidental in relationship. Carotene may, however, act as a light-screen, thereby enabling a well-defined spectral field to initiate synthetic change. Whether or no cholesterol is derived from carotene, phytosterol or from protein, it possesses well-marked biological characteristics. Although its molecular structure has not yet been completely elucidated, it is known that it contains a hydroxyl group and a double bond, both of which are intimately associated with the integrity of its biological function. Interference with one or the other or with both of these factors, or interference with its optical polarity, brings in its train a paralysis of its anti-hæmolytic function. Chemically, it is a comparatively inert lipin alcohol, and in virtue of its high degree of insolubility in aqueous fluids, it is easily thrown out of solution. Deposits of cholesterol and cholesterol esters are accordingly found in cystic fluids, and in centres of active adsorption, such as atheromatous patches, inspissated pus collections, old infarcts, hæmatomas, etc. Dewey (1919) reported that the predominant lipin in jaw tumours is cholesterol. Powell-White (1908) has made an extensive study of its distribution in tumours. He invariably found crystals consisting of a loose combination of cholesterol with fatty acids and lecithin. These crystals seemed to be associated with cell proliferation rather than with degeneration.

The ease with which cholesterol is adsorbed from circulating blood is partly accounted for by its insolubility and lack of reactivity. The crystals of a tumour mass are partly derived from precipitation supersedent to autolytic breakdown within the tumour, partly from external sources by adsorption. Adsorption is, however, counterpoised by lymph and blood uptake to such a degree that one would not expect from this source an explanation of the fluctuant values obtained in Tables I, II, III and IV. Besides, the fluctuation is regular and seems to be independent of the nature of the tumour, whether carcinoma or sarcoma, of its duration, rate of growth, or of the age of the patient.

In Table I, where cholesterol values are given for patients in the winter months, there is a definite average value of 0·077 per cent. for the plasma and corpuscular distribution of this substance. In spring the value changes gradually to 0·14 per cent., and in the summer months the maximum concentration of 0·22 per cent. is attained. There is a pronounced fall in autumn to 0·15 per cent.—approximately the spring value. The corresponding normal values are slightly higher, which finding implies the presence of a seasonal variation of cholesterol in normal blood. These corresponding average values are 0·11 per cent., 0·18 per cent., 0·28 per cent., 0·18 per cent. *There is thus indicated a slight hypocholesterolæmia in malignant disease.*

If, as Dorée suggests, the cholesterol of the body is derived from plant sterols, then the graduated change in vegetable consumption during the course of the year may largely influence the gross value of circulating cholesterol. The values found at various seasons, however, show remarkably distinct lines of demarcation, which are not easily explicable on the basis of a graduated yet irregular change in vegetable uptake in the animal economy.

Some evidence has been adduced that solar radiation is the cause of cholesterol change, that the sun, acting upon peripheral centres of synthesis, initiates tissue changes which favour either an accelerated transport of mobilizable cholesterol or an autochthonous synthesis of that substance, which then traverses the capillary network and is taken up in the systemic circulation.

SUMMARY.

In normal blood there is a seasonal rhythm in the cholesterol concentration. In malignant disease there is a similar periodic change in cholesterol content. From the data given in this paper, the marked seasonal parallelism would indicate a slight hypocholesterolæmia in malignant disease.

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LOCAL AND GENERAL DEFENCES AGAINST INFECTIONS, AND THE EFFECT ON THEM OF VITAMIN-DEFICIENCY.

W. CRAMER AND A. NEAVE KINGSBURY.

From the Laboratories of the Imperial Cancer Research Fund, London, and the Bland-Sutton Institute of Pathology, Middlesex Hospital, London.

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ONE of the most constant effects of a diet deficient in vitamin-A is the development of bacterial infections of an avirulent type. The most frequent and most obvious infection is xerophthalmia. Sometimes there is a subcutaneous abscess in the neck. When vitamin-A is again supplied these infections rapidly disappear. The following investigation had as its object to determine on what changes the diminution in the resistance against these infections depends.

The following observations were carried out at the same time as those on radiated rats reported recently by one of us (A. N. K.) in conjunction with J. C. Mottram (Mottram and Kingsbury, 1924), and the technique used was in every respect the same, so that no further details are given in this paper. It has been shown in previous papers that there is an essential similarity in the effects produced by vitamin deficiencies on the one hand and by exposure to X rays and radium on the other, and this finds further confirmation in the results of the present series of observations.

Blood-cultures.—Seven vitamin-deficient rats were examined. Four gave positive (heart) blood-cultures (1 *S. albus*, 1 *S. aureus*, 1 streptococcus, 1 pneumococcus). Three were negative. Five normal controls gave four sterile blood-cultures, one positive (*S. albus*). Anaërobic cultures proved to be sterile in every case. Coliform bacilli, which were found in radiated rats, were not found in vitamin-deficient rats.

Agglutinins.—In spite of the absence of coliform bacilli, the vitamin-deficient rats developed in their serum an agglutinin against *B. coli*, while the serum of radiated rats, like that of normal rats, does not contain such an agglutinin. For the demonstration of this agglutinin an emulsion of 500 million organisms per c.c. was used. Tubes incubated at 37° for 1 hour and read after standing for 1 hour at room temperature. The organism had been isolated from the heart-blood of a radiated rat.

Serum dilutions.

		$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$
Vitamin-deficient rats	.	1·4	4	4	3	1
	.	2·4	4	4	3	1
Control rats	.	1·0	0	0	0	0
	.	2·0	0	0	0	0
	.	3·2	1	0	0	0

4 = complete sedimentation. 3 = large flocculent masses with sedimentation incomplete. 2 = fine flocculent masses. 1 = trace of flocculation only.

The reasons for this difference between radiated rats and rats kept on a vitamin-A-deficient diet is that in the latter case the changes take several months to develop, while the effects of radiation are acute and develop with a sufficiently massive dose in a few days. The development of an agglutinin for *B. coli* on a vitamin-A deficient diet not only explains why coliform bacilli are absent from the blood, although the intestinal atrophy after vitamin-deficiency is essentially the same as after radiation, but is in itself evidence that a leakage of coliform organisms into the tissues occurs.

The further conclusion, that vitamin-A deficiency does not inhibit the development of agglutinins, was confirmed by experiments with *B. typhosus*.

A laboratory strain of *B. typhosus* was used. An emulsion of 50 millions per c.c. from a 24 hours' culture killed at 55° C. for 30 minutes was inoculated intraperitoneally into the rats in doses of 0·5 c.c. The inoculation was repeated 4 days later. Blood for the agglutination test was taken 8 days after the second inoculation. The same emulsion of the same culture was used for the test.

Serum dilutions.

		$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$
Vitamin-deficient rats :					
1. *minus* A ; *plus* B	.	4	1	0	0
2. „ A ; „ B	.	4	3	1	0
3. „ A ; *minus* B	.	3	2	1	0
4. „ A ; „ B	.	4	3	2	1
5. „ A ; „ B	.	4	3	2	1
Control rats :					
1. *plus* A ; *plus* B	.	4	2	1	0
2. „ A ; „ B	.	4	3	1	0
3. „ A ; „ B	.	4	3	2	1

The experiment was repeated on four other rats kept on diets free from vitamin-A with identical results. In this respect there is complete agreement with the effects of radiation. Observations on four vitamin-deficient rats showed that this also applies to the hæmolytic complement, which is unaffected both by vitamin-A deficiency and by radiation.

Fragility of blood-corpuscles.—Since there is frequently an anæmia in rats kept on a vitamin-A-deficient diet—as there is after exposure to radiations—the fragility of red blood-corpuscles was determined. Five vitamin-deficient rats, like the radiated rats, showed no difference from the normal. The anæmia is therefore not due to an increased fragility of the red cells.

General resistance to infection.—Inoculations with different bacteria (*B. anthracis*, streptococci, *B. coli*) did not reveal any obvious diminution in the general resistance of the vitamin-deficient rats. The only exception was found in infections with *B. tuberculosis* (bovine type). Rats are normally immune to this infection. But two vitamin-deficient rats injected intraperitoneally with 1 c.c. of an emulsion of 100 million bacteria per c.c. showed when killed four weeks later enlarged mesenteric glands which gave positive smears. In two normal control rats which had received the same injections the glands were not enlarged. A similar effect had been observed in radiated rats (Mottram and Kingsbury, 1924).

DISCUSSION.

The observations show that a deficiency in vitamin-A does not affect appreciably the general humoral defences against many bacterial infections. Now one characteristic feature of the bacterial infections resulting from vitamin-deficiency is that they are localized and always affect the same organs. The eye is the most obvious but not the only organ affected. We have shown in previous papers and illustrated by drawings (Cramer, 1923; 1924) that the intestinal mucosa itself gets infected by the intestinal bacteria. This is a local infection in the sense that acne and impetigo are local infections of the skin. In a normal animal the intestinal bacteria are restricted to the central lumen of the intestine. In a rat kept on vitamin-A-free diet—and the same applies to a radiated rat—the intestinal bacteria penetrate between the villi into the crypts of Lieberkühn and into the mucous glands of the cæcum, which they fill, and where they proliferate. In the eye this local infection is associated with the atrophy of one of the para-ocular glands, in the intestine with a similar atrophy of the mucous glands. We may conclude from this that these local mechanisms of defence against bacterial infection are represented in the eye by the secretion of the para-ocular glands and in the intestine by the secretion of mucus, and that the local infections occurring in vitamin-A deficiency are due to the atrophy of these local tissue defences. This conclusion is confirmed by the findings of Mori (1922), who, in vitamin A deficient rats, has observed an atrophy of the mucus secreting cells in the salivary glands and in the mucous membrane of the trachea and larynx. This was followed by a local bacterial infection of these tissues.

The frequency with which the intestinal canal is infected by nematodes, cestodes and protozoa in rats kept on a vitamin-A-free diet is probably due to the same atrophy of the mucous secretion.

There is a third organ which is frequently the site of bacterial infections in vitamin-A deficiency—namely the lungs. When rats are kept for a sufficiently long period on a diet free from vitamin-A and this diet is continued after the typical xerophthalmia has developed, the rats die as a rule with a septic broncho-pneumonia. These pulmonary infections develop much more slowly, but they do not require the complete absence of vitamin-A for their occurrence. They have been a frequent occurrence in the older rats of our ordinary laboratory stock as long as they were kept on the vitamin-poor diet of bread, maize and rice which used to be the ordinary laboratory diet. They

have become rare since this diet was enriched with vitamins. Rats which have been subjected to a vitamin-A deficiency and, after having been "cured" from their xerophthalmia by cod-liver oil, have been kept alive on a diet which is not very rich in vitamin-A, will in our experience almost invariably develop this septic broncho-pneumonia if they are kept alive long enough. The point is of clinical importance, because the reports of the various Danish authors on the epidemics of xerophthalmia in children due to faulty diet show that in the fatal cases lung infections were the cause of death almost invariably. And when McGowan and McNeil (1913) studied a similar epidemic in this country at a time when the importance of vitamins was not understood, they described it as a "distorted pneumonia." At present it is taken for granted that an animal can be "cured" from the effects of vitamin-A deficiency by an ample supply of cod-liver oil, the criterion being the disappearance of xerophthalmia. Our observations suggest that an organ such as the lung cannot always rid itself completely from bacteria once they have been allowed to get a sufficiently firm foothold. The experiments with tubercle bacilli suggest that local defences are of greater importance in conferring resistance against this infection.

Under the ordinary conditions of vitamin experiments the breaking down of local defences allows access to the comparatively avirulent bacteria which normally inhabit the intestine, the respiratory tract or the conjunctival sac, so that the resulting infections are as a rule avirulent in type. When organisms of a more virulent type are present, the condition of the local defences rather than the mere presence of these organisms may be the factor which determines the onset of a virulent infection. Such an occurrence is reported by McCarrison (1922). He found amœbic dysentery occurring in monkeys kept on a vitamin-deficient food, while the control monkeys kept in the same room and fed by the same attendant remained free from it.

The significance of the thrombopenia observed in vitamen-A deficiency (Cramer, Drew and Mottram, 1923) has been discussed in a recent paper (Cramer and Drew, 1923). It is caused by the steady invasion of bacteria when the local defences are broken down, the platelets attaching themselves to the bacteria. The thrombopenia of vitamin-A deficiency is therefore the result of the bacterial invasion, and does not necessarily indicate a weakening of the mechanism of defence represented by the platelets, as was originally suggested. It is different with the thrombopenia of rats kept in the dark for long periods. This does represent a weakening of the mechanism of defence, since there is no bacterial invasion to account for it. Rats kept in the dark do not exhibit the spontaneous development of infections characteristic of vitamin-A deficiency, because absence of light does not produce the lesions, intestinal and otherwise, which impair the local defences. But when such rats are also subjected to the vitamin-A deficiency the infections develop more rapidly, because in addition to the breaking down of the local tissue defences by the vitamin deficiency, there is the weakening of the general defences represented by the thrombopenia of darkness. Conversely exposure to light, by stimulating the production of platelets, delays the onset of the bacterial infections.

Bannerman (1924) has recently published an interesting observation on

the human subject with reference to the effect of a local infection of an avirulent type on the blood-platelets. A subject on whom daily platelet-counts were being made developed a cold: at once the platelets diminished in number; their number increased again to the normal as the cold passed off.

CONCLUSIONS.

In addition to the general humoral defences against infection, there are local defences for certain organs, such as the intestine, the eyes and the lungs. A diet deficient in vitamin-A impairs the efficiency of the local tissue defences. It does not appreciably diminish the efficiency of the general humoral defences. The study of these agencies emphasizes the importance of the local defences by showing that their breaking down will allow infections to set in even though the general defences remain unimpaired.

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